uc3m | Universidad Carlos III de Madrid

University Degree in Biomedical Engineering
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Bachelor Thesis

"Designing a system for recording fluorescence photometry in awake animal with freedom of movement"

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Abstract

Current advancement with optogenetic probes for imaging and manipulating neural activity has further extended the relevance of fiber-optic systems for neural circuitry research. Optical fibers, which bi-directionally transmit light between separate sites, can be used for either optical imaging or manipulating neural activity relevant to understand the circuitry mechanisms that underlying the behavioral. Therefore, optical fibers have many usages and, one of them, is intracranial photometry through chronically implanted optical fibers, which is a broadly used technique for measuring signals from fluorescent probes in deep-brain structures. Additionally, the recent proliferation of bright, photostable, and specific genetically encoded fluorescent reporters for calcium and other neuromodulators has dramatically increased the utility and popularity of this technique. The main objective of this thesis is to build a system of fluorescence photometry using a recycled microscope. For its development, many changes are performed to the microscope in order to adjust it for optimal using with the system. It is connected to the LED source, the optical fiber, and the photomultiplier. Also, the LED is driven by ARDUINO circuit to control the frequency and blinking time of the light, and to a potentiometer to control the intensity of the light. The picked up signal with the photomultiplier is amplified, filtered, and finally acquired by an ADC data acquisition system.

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Acronyms

LED: light emitting diode

NA: numerical aperture

PMTs: photomultiplier

IDE: integrated development Environment

SNR: single to noise ratio

eGFP: enhanced green fluorescent protein

AAV: Adenosine associated virus

1.Introduction:

1.1. Motivation

Neuroscience includes a wide-ranging question about the way the nervous system of humans and other animals works, how it is responsible for feeling, sensing, making decisions, and taking actions. In order to find answers to all these questions, researchers and scientists have been studying the brain for centuries. Along with the advancement of technology during the 20th century, neuroscientists could take zoom in on the brain to understand its complexity. Specialized cells, called neurons, are connected and form circuits to link different regions of the brain. Apart from this, neurons act differently during diseases. There are plenty of tools to study the brain, like behavioral observation, anatomy, psychophysics, brain functional image, and electrophysiology. However, in this project, we are interested in another tool, which is optogenetics.

Optogenetics is considered one of the most modern tools in the neuroscience field and it has changed and revolutionized the way we can study the brain. With these new techniques, we can study any brain region or any neuron circuit by turning on and off certain cells in specific regions. Additionally, we can see whether or not they have a rule in a specific function or if they go wrong during certain pathology. Indeed, to do this, we need two main things: light and small molecules, that can bind to a different type of neurons without harming them, and able to transduce light into electric signals (fluorophores).

In this project, I am designing a fluorescence photometry system to study a live animal brain by using a recycled fluorescence microscope. Taking into mind that this system has to be flexible and can be used to study different neurons as well as that it can be modified and enhanced in future projects.

1.2. Techniques used in experiments with animals

1.2.1. Fluorescence microscope

Fluorescence microscopy is widely used in biological investigations. Its use is expanding in all fields of molecular and cell biology. It has plenty of applications including monitoring living cellular activities with the help of genetically engineered fluorescent proteins.

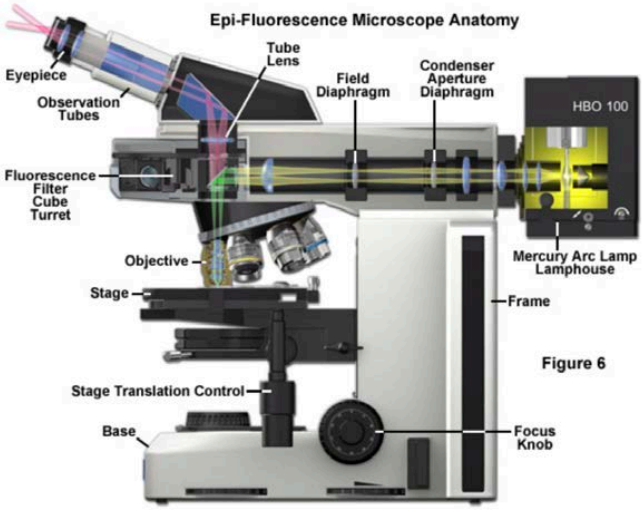


Figure 1: Fluorescence microscopy anatomy [1]

The way the fluorescence microscope works is by letting excitation light radiate the sample and then filter out the lower energy emitted light from the specimen. This filtration is done with the help of excitation and emission filters in addition to a dichroic mirror. First, the excitation filter only lets the light within a specific range of wavelengths. This range should match the fluorescing material (fluorophore excitation wavelength). Then, the light photons will hit the atoms of the specimen, which leads the electrons to excite to a higher energy level. Once they relax to a lower level, they will emit light with a longer wavelength (lower energy). This light is going to pass by the emission filter, which will separate the specimen's emitted fluorescence from the excitation light. Finally, the light will go to a detector allowing us to see the image.

Usually, the molecules which emit the light are fluorophores. They are used due to their behavior when subjected to photons (light). Both, the wavelengths of absorption and emission and the fluorophore efficiency as a fluorescent compound, are determined by its outermost electron orbitals. At rest, the fluorophore is in the ground state. However, once it absorbs light energy, variations in the vibrational, rotational and electronic states of the molecule may occur because electrons will move into a farther orbital from the nucleus, and the fluorophore becomes excited. This transition is very fast, from the order of femtoseconds. Eventually, the absorbed energy is shed, leading the fluorophore to return to its ground state. Figure (2) illustrates the times that various steps in fluorescence excitation and emission take.

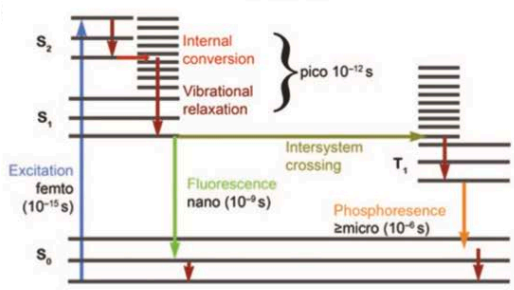


Figure 2: fluorescence fundamentals [2]

A crucial point to keep in mind when choosing the components of the filter cube (Fig. 3) is the difference between the exciting and emitted wavelengths, known as the Stokes shift. The better the Stokes shift, the more powerful the fluorescence (in optimal cases, there would be complete filtering of the exciting light without preventing the emitted fluorescence from reaching the detector).

Another critical point is that the fluorophore emission wavelength is going to be always higher than the excitation wavelength because there is energy loss through stokes shift. In other words, emission light energy is going to be lower that excitation light energy (Fig. 4).

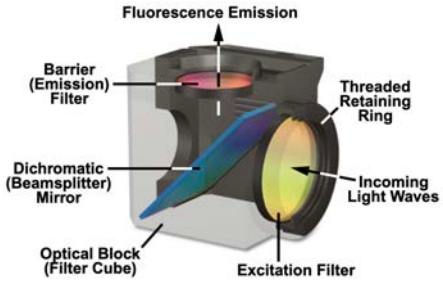


Figure 3: fluorescence filter [3]

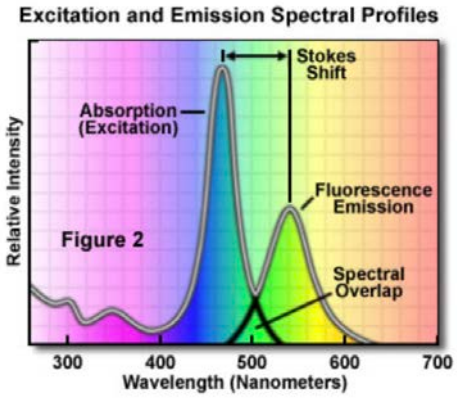


Figure 4: Excitation and emission spectral profile [4]

1.2.2. Confocal

In recent years, confocal microscopy became one of the most popular techniques used in optical images as it offers many advantages that made it more preferable to use than conventional widefield microscopy. These advantages, including, the ability to reduce or eliminate background information away from the focal plane, the ability to collect serial optical sections from a thick specimen, and the ability to control field's depth. The main idea behind the confocal technique is eliminating out focus light in a sample whose thickness surpasses the immediate plane of focus by using special filtering techniques, allowing us to obtain high-quality images. Actually, with the increasing number of applications in cell biology that depend on imaging both living and fixed tissue, confocal microscopy is demonstrating day after day to be on the most significant achievement in optical image approaches.

To understand the way how it works, we need to know its most essential components. The scan head, which is illustrated in figure(5), is at the core unit of the confocal system, it rasterizes the excitation scans and collects the photon signal from the sample to form the final image.

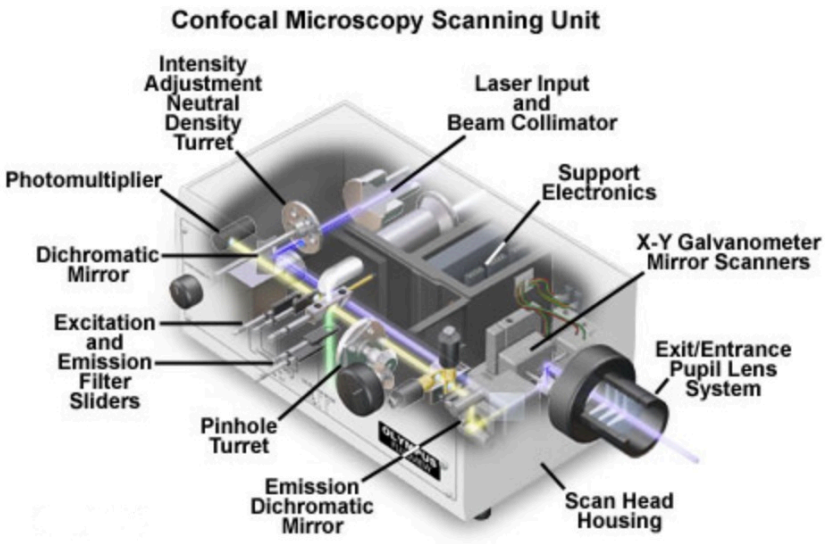


Figure 5: confocal microscopy scanning unit [5]

Unlike the fluorescence microscope which uses a high-intensity light, the Confocal microscope uses a laser as the excitation source. Images are taken with a digital camera with a pinhole. This feature, allows the light of only one focal plane to be focused on the digital camera, the light waves coming from above and below focal plane are canceled out.

The way that confocal microscopy works (Fig. 6) is explained in the following steps:

1- the laser focuses on a tiny spot of the specimen, with the help of the lens system, resulting in illumination point size between 0.25 to 0.8 µm in diameter (depending on the objective numerical aperture) and 0.5 to 1.5 µm deep at the high intensity. Then, the fluorescent dye emits a fluorescence which is captured by a digital camera.
2- by using two rotating mirrors in X and Y directions, the laser is now focused in the next region of the specimen and fluorescence is captured by the digital camera.
3- the laser scans the whole surface of the specimen, and images from each spot are captured.
4- a software combines all of these images in one sharp image.

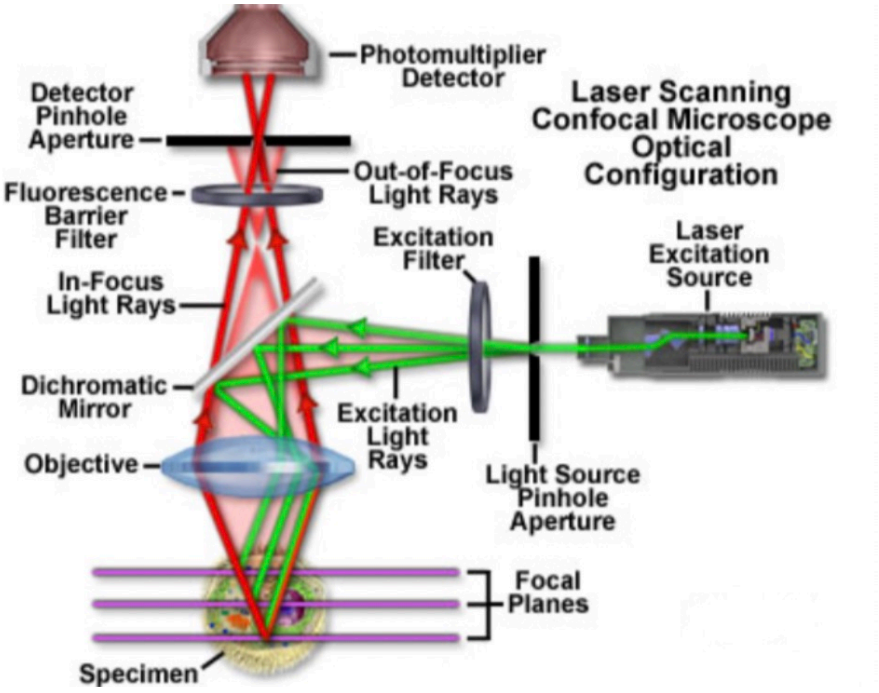


Figure 6: confocal microscopy anatomy [5]

1.2.3. Two-photon microscopy

Two-photon microscopy is a special technique, in which two photons are used to excite one electron in the ground state. The optical scheme of the two photons microscope is similar to the confocal one with the main difference: the illumination source and the lack of the sample pinhole [6].

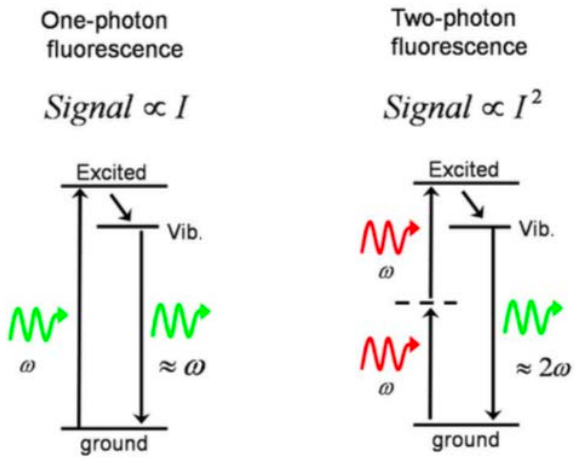


Figure 7: Electron excitation in confocal (left) and two-photon (right) microscopes [6]

One of the weaknesses of confocal microscopy is the limitation of the imaging depth due to two main reasons: physical limitation (objective working distance) and tissue penetration depth (in the real world, thick tissues absorb and scatter light). To overcome these obstacles, two photon microscopy uses laser light to excite the fluorophore with twice the excitation wavelength (far-red, infrared), which will enable the light to reach deeper regions in the tissue. Furthermore, "Longer wavelengths are also less toxic for cells and moreover, the probability of secondary photochemical reactions is significantly much lower than that observed in confocal microscopy" [6]. Figure (8) shows the relation between penetration depth and light wavelength.

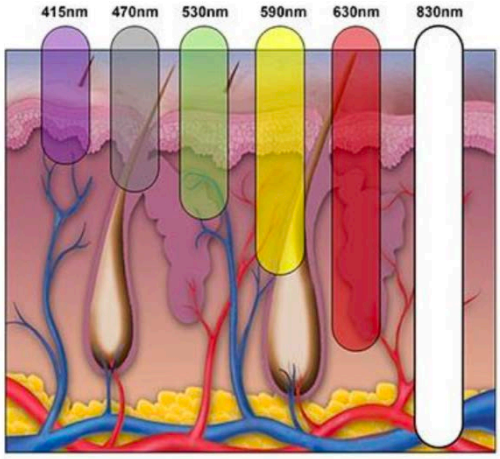


Figure 8: tissue penetration for different wavelengths

In addition to that, most of the fluorescence produced in the Z axis by confocal microscopy is concentrated at the focal plane, while two photon microscope has the ability to produce fluorescence in a very narrow spot (surrounding tissues do not experience photodamage). This is illustrated in figure (9).

Also, unlike the confocal microscopes in which the laser emission is constant, two-photon microscopes use pulsed laser to concentrate photons as much as possible in every single pulse.

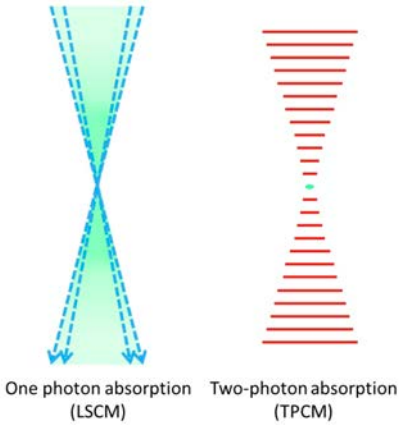


Figure 9: fluorescence producing in confocal (left) and two-photon (right) [7]

1.2.4. Fiber photometry

Fiber photometry is one of the most popular methods in neuroscience since it is used to record genetically defined neuronal and glial cell populations in live animals and tissues.

The basic concept behind this method is that the excitation light passes through a single or multimode optical fiber, either directly implanted or coupled to an implanted probe or cannula in the brain (Fig. 10) [8].

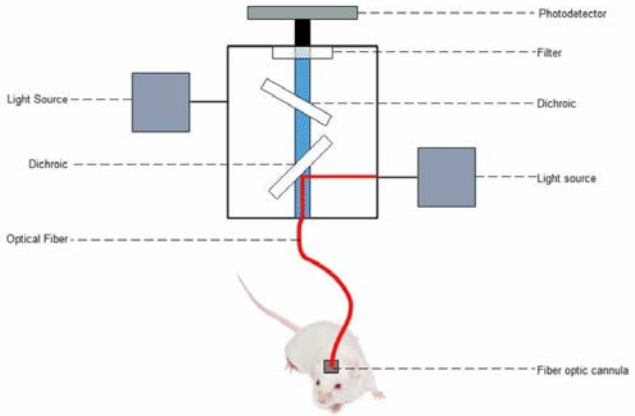


Figure 10: principle of fiber photometry [8]

The excitation light will first pass through the excitation filter, then a dichroic mirror will reflect it into the optical fiber. This light can activate or silence genetically encoded or injected reporters (fluorophores) in a group of nerve cells and monitoring their activity. Once the fluorophore is excited, it will emit light with a higher wavelength that will be transmitted back through the same fiber and detected by CCD camera, photodiodes, or photomultiplier (PMTs). A dichroic mirror separates it from the excitation light before reaching the detector. In the case of PMTs, it will measure fluorescence variation signal and transfer it into a current amplifier, which will amplify this signal before a data acquisition system can record it.

2.1.3.1. Advantages of fiber photometry

There are many advantages for using optical fiber in optogenetics over other techniques [9]:

- It allows deep penetration for the light and can be used to study the activity of deep regions of the brain like the hypothalamus.
- The small diameter of the unit core of optical fibers usually used in photometry systems (200-400 um), allows monitoring activity in tiny specific regions. Also, it causes less invasiveness compared to other methods that use implants like micro-lenses.
- They can be used in areas with electromagnetic interference where electrical -based sensors could fail since they are not affected by EM interreference. Also, they can be used in conditions with high temperatures.
- Availability of a wide range of optical fibers that exhibit low attenuation over a large wavelength range.
- Optical fibers are easily embedded to materials, thanks to their uniform cross-sections and relatively small dimension
- Its lightweight (usually below 10g) allows using it in freely moving animals' studies since the weight load on the animal's head is minimal.
- No heat generated near the tissue since there are no electrodes used.

1.3. Importance of Calcium signal in neuroscience

Calcium imaging is one of many approaches in monitoring the activity of the entire population of neurons. The main principle of this approach is recording the transient changes in the intracellular concentration of calcium ions [10]. These ions are associated with action potential initiating in the myocardium cell, since they are one of the most common intracellular messengers in neurons, and their channels start currents that lead to voltage changes in neurons [11].

Ordinarily, the concentration of calcium ions (Ca^{2+}) in the cytosol is extremely low, typically 50 to 100 nanomolar (10^{-9} *M*). While, the concentration of (Ca^{2+}) ions outside neurons—in the bloodstream or cerebrospinal fluid, for instance—is several orders of magnitude higher, typically several millimolar (10^{-3} *M*) [10]. This deep gradient is maintained by many mechanisms like calcium pumps or sodium-calcium exchangers.

However, this concentration is not constant. When a legend binds to a calcium channel, it opens, allowing calcium ions (Ca^{2+}) into the cell. The same phenomena happen when voltage-sensitive calcium channels open after being affected by a voltage change across the membrane. This transient rise in cytoplasmic calcium concentration transmits information within the cell. This rise allows calcium ions to bind to a large number of calcium-binding proteins that serve as molecular targets and can initiate a variety of activities.

The rise in calcium allows calcium ions to bind to a large number of calcium binding proteins that serve as molecular targets and can initiate a variety of activities. For instance, when calcium ions bind to Calmodulin, they will be activate it, which in turn (activated Calmodulin) will activate downstream targets such as protein kinases.

This ability of calcium ions to produce multiple intracellular signals that control key functions in all types of neurons and the fact that calcium signals have particular functions in well-defined cellular sub-compartments, made developing of technologies for calcium imaging highly demanded [11].

When scientists started measuring Ca^{2+} signals, they used isolated living cells and cells in tissue culture. However, in order to describe systems level physiological regulations, it is crucial to understand the signal flow between cells and intact systems. This is very complex to be done and cannot be accurately reproduced in *vitro*. Therefore, scientist tended towards recording and analyzing integrated signals between cells in living tissue in *vivo* [12]. (Fig. 11)

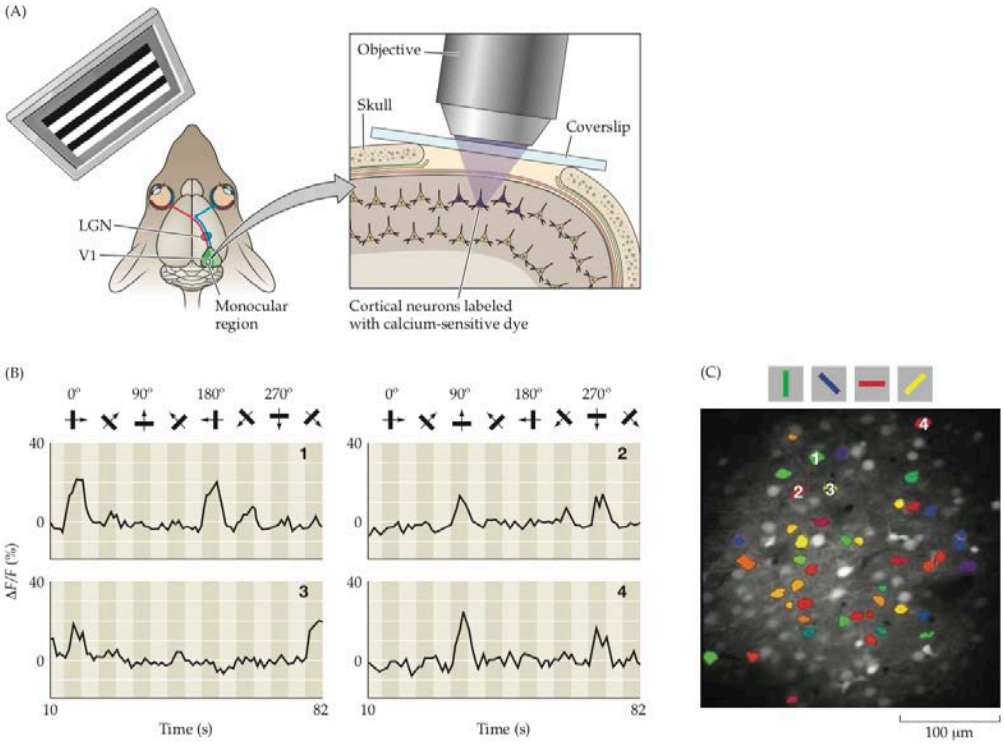


Figure 11: imaging cortical neurons responding to a visual stimuli using calcium sensitive dyes [10]

1.4. State of art

The development of photometry systems new techniques has been increased scientifically in recent years. This is due to their crucial role in helping scientists understand the brain function

Photometry systems that use optical fibers have some "physical constraints induced by the cables and the bulk, size, and weight of the associated fixtures complicate studies on natural behaviors, including social interactions and movements in environments that include obstacles, housings, and other complex features" [13] .Thus, a group of scientists has developed a novel system, in which stimulation and recording of the fluorescence signal are wireless.

In this system, a photodetector and a miniaturized light source are integrated into an injectable fluorescence photometer which is covered by a polymeric layer that made it biocompatible so that it can be injected into sites of interest in deep regions of the brain. Figure (12) illustrates the principles of this novel method.

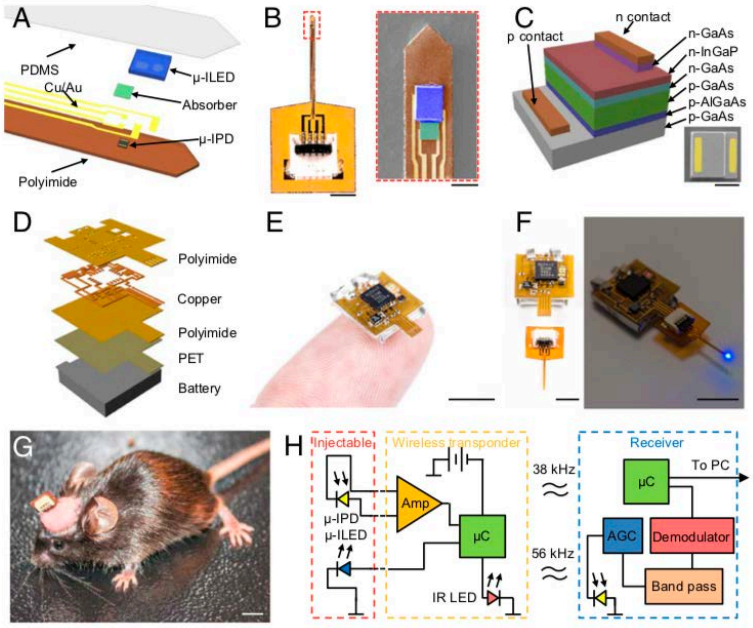


Figure 12: Wireless optoelectronic photometers [13]

In order to test its reliability, the system was used to record the calcium activity in deep brain. The results were similar or even better than those provided by conventional fiber photometry systems (control system). This high-fidelity recording results as well as the capability of studying freely moving animals without objecting them to the optical fiber additional weight make this new method to be considered as a potential candidate for an extensive use in neuroscience research.

1.5. Legal frame

During the project, and since there are other machines and devices in the lab, an exterior residual current device was used to break the circuit in case there is a mistake in the connections. Shape adjustments were done in the technical room after taking all necessary cautions. Regarding the wires, their sizes were chosen in an appropriate way so no overheating or energy dissipation may occur. Also, they were all thermally insulated.
This project took place at Cajal institute of neuroscience, CSIC. And the using of animal fulfilled all the requirements the agreement on transparencyon animal research [14]. Moreover, "the Cajal Institute ensures that the personnel involved in animal care and researchers do have the adequate education and training and the required professional skills, and that all resources are provided to properly keep research animals in terms of facilities, husbandry, wellbeing and veterinary care" [15].

1.6. Objectives

This bachelor thesis is about modifying a recycled fluorescence microscope and adjusting it into a fluorescence photometry system to use it in studying animals' brains.

This system will record intracellular calcium activity in specific regions of the animal brain (depends on where we implant the cannula). Recording the intracellular calcium and other second messengers give an important physiologic measurement and will help in clarifying the fundamental biophysical and molecular mechanisms which are currently at the top priority of neuro and cellular research.

The goal behind this project is to construct the system from scratch and after this to test it in order to see its functionality. The development of this system has many tasks:

1. Commissioning of high intensity LED kit.
2. Modulation of intensity and frequency of light emission.
3. Adjustment and calibration of the photomultiplier.
4. Acquisition and analysis of the signal emitted by the photomultiplier.
5. Design and assembly of the different parts of the photodetection system.

This general objective can be divided into the following specific objectives:

1. Understanding the individual components of the device and how they work.
2. Assembly of the different components.
3. Make proper adjustments to the microscope.
4. Make proper connections to synchronize the system.
5. Write an initialization control to facilitate device use for other users.
6. Testing of the system.

2. Materials and Methods

2.1. System components

In order to be able to understand the how the whole system was implemented, an explanation of the single components and how they work together is required. In order to be able to acquire accurate results, precise coordination of the different components is needed.

2.1.1. LED kit

The LED kit DK-114N-1 from LIMINUS was purchased and then assembled. It has many features which made it an optimal option for using with any PT or CBT series big chip LEDs and driving them, it;

- Can handle a pulse frequency of greater than 40 kHz
- Can drive big chip LED up to 14 A which assure high brightness delivery.
- Have fast rise and fall times (less than 1 microsecond)

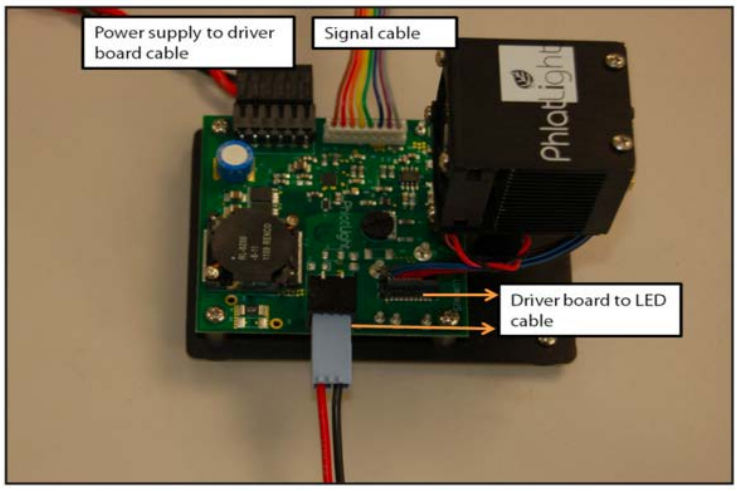


Figure 13: completed electrical connection on DK-114N-1

In Addition to that the development kit can drive LEDs in continuous-current control either through an external analog voltage or via on-board POT, signal PWM dimming is available by using an external function generator or a microcontroller.

In this project, I decided to control the LED signal by ARDUINO, since both the magnitude of the signal (brightness of the LED) and blinking period can be handled and modified simply. This was done by connecting the 0-5 V pulsed output of the ARDUINO with the PWM and GND pins of the signal cable of the LED.

First thing I have done was to mount the components of the LED, then connect it to a converter (220-12 V). In order to test if the LED is working properly, I sat the value of the on-board POT at the maximum (10kohm), then I started decreasing its value while watching the brightness of the LED. The brightness of the LED was increasing while the value of the POR was decreasing.

However, in order for the LED light to reach the excitation filter with no attenuation or distortion, I had to fix it in a place where it could be close to the filters cube. So, I have done some modifications taking advantage of some pieces from the old microscope (Axioscop 2 FS Plus, Zeiss, Germany).

The following photos illustrate some of the done work (see figure 14):

- adjusting a hole in a piece of metal that used to be in the backside of the microscope to fit precisely to LED cube. Thus, the LED can be placed as close as possible to the rest of the system.

- The cables of the fan, which are used to cool the LED, were elongated by using similar wires because they were too short and prevented the ability to place the LED away from the driving circuit.

- Four holes were drilled inside the body of the microscope in order to fix the driver board, it had to be as close as possible to the LED since the driver board to LED cable is kind of short.

- A small square hole was drilled to enable the signal cable to reach the Arduino circuit, which is located on the other side of the microscope.

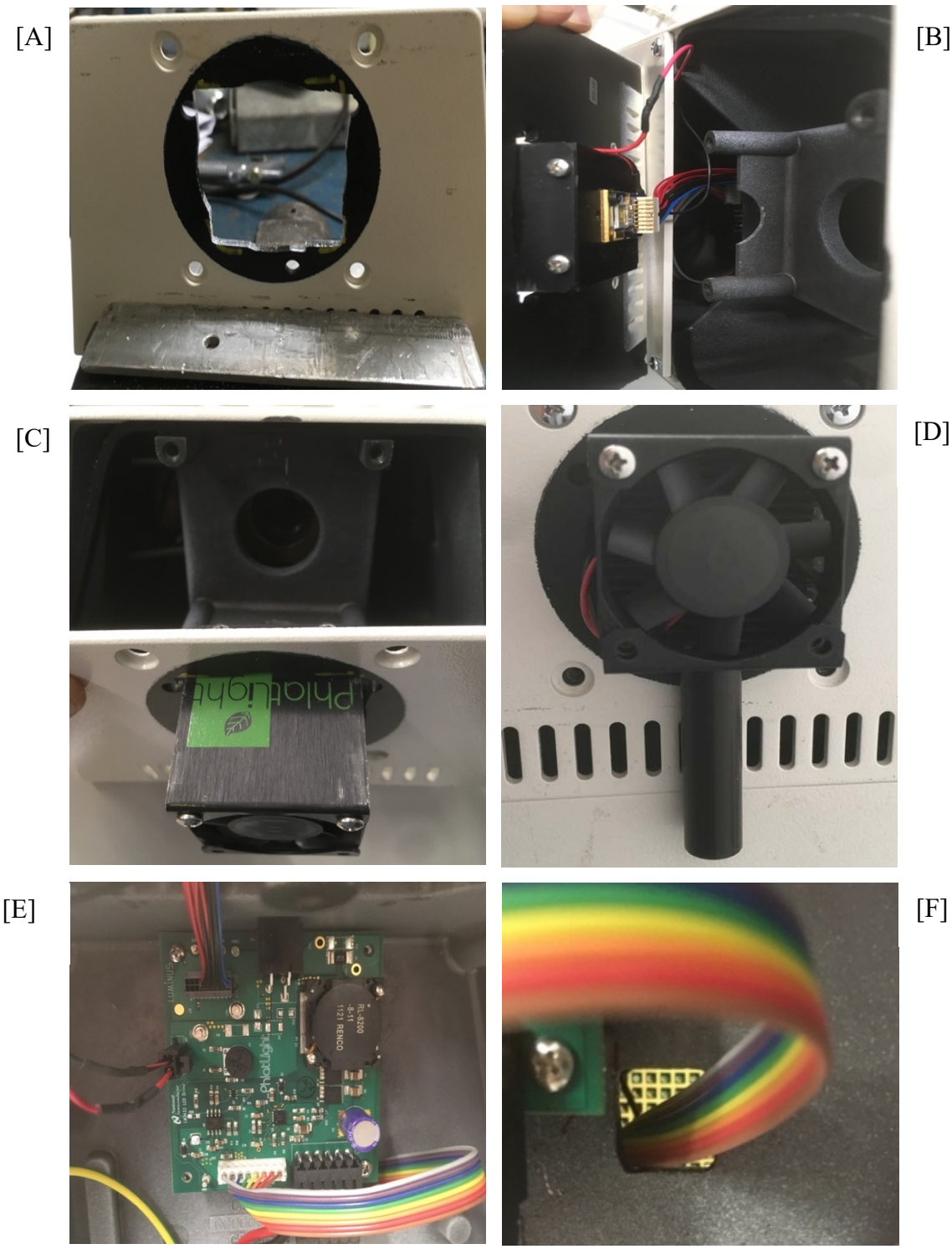


Figure 14: (A) adjusting a metal piece to fix the LED at its position, (B) thermally insulated elongated cable. (C) LED placement at the closest point to the rest of the equipment. (D) fan position with a free space behind it in order to work properly. (E) LED driver circuit. (F) LED signal cable.

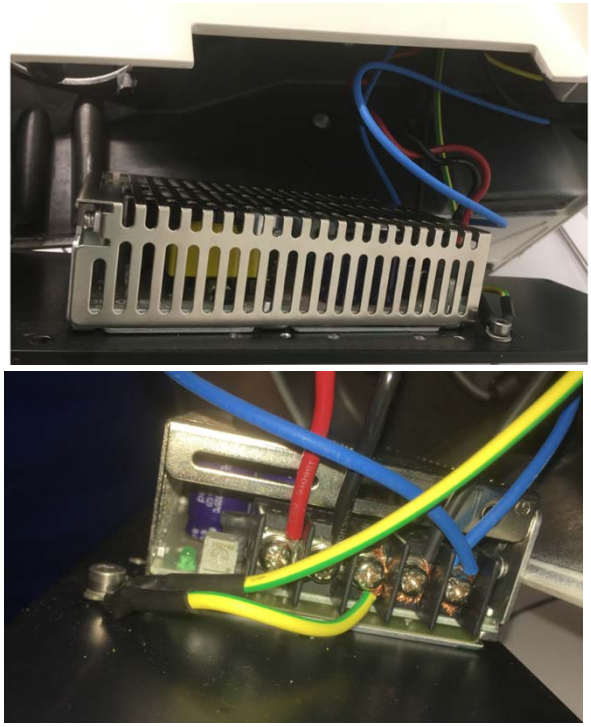
2.1.2. Converter

A power supply with isolated outputs is required to properly drive the common anode LEDs, and failure to use an isolated power supply may damage the driver board. For best efficiency, (SPN5-12S, KGCOMP) was chosen. It has an enclosed mounting design and the following characteristic.

MODEL	SPN50-12S	
AC Input Voltage	115V	230V
Output Voltage	12	
Output Current	4.3	5

Table 1: converter characteristics

For system designing purpose, four holes were drilled in the base of the microscope in order to fix the converter within the device. (Fig. 18)



[A]

[B]

Figure 15: (A) converter placement at the microscope base. (B) inputs and outputs of the converter

Before connecting it to the LED kit and in order to protect the LED from excessive voltage draw, the output of the converter was tested. The values were similar to characteristic one.

2.1.3. ARDUINO

ARDUINO allows us to implant PWM in several different ways. By modifying the duty cycle of the clock, the pulse width of the signal can be modulated, which results in controlling the brightness of the LED.

The main idea of PWM is getting a square digital wave with the ability to control its width by turning the signal on and off. Therefore, by changing the portion of off time and on time, we can get different values of voltage between minimum and maximum values. In Arduino case, between 0 Volts (off) and 5 Volts (full on). Thus, we can control the brightness of the LED. On other words, we can control the duty cycle, which represents how much of the period the period in which the signal is high. This is illustrated in figure (16)

To calculate the duty cycle:

$$duty\ cycle = {Ton}/{Ton + Toff} \quad \text{..... (1)}$$

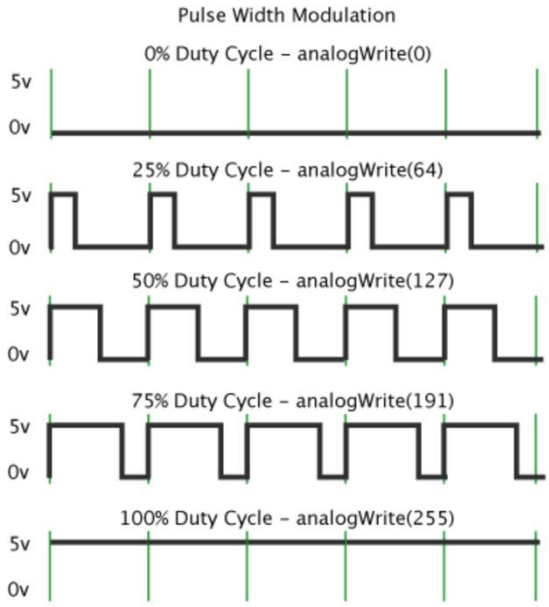


Figure 16: Pulse width modulation [27]

Arduino's PWM frequency is controlled by the timers of the Atmega microcontroller and it is normally set at constant value for each pin. In our case, pin 9 generates PWM at frequency of 490 Hz, this means it has a period of almost 2 milliseconds. However, we can set this frequency by changing the pre-scalar of the timers and effectively change the frequency. We can also download a library for PWM and call a function (SetPinFrequencySafe()) which will allow us to change the PWM frequency in the Arduino IDE.

Another important point is relating ADC value to voltage, this can be done using the following formula:

$$\frac{\boldsymbol{Resolution\ of\ the\ ADC}}{\boldsymbol{system\ Voltage}} = \frac{\boldsymbol{ADC\ reading}}{\boldsymbol{Analog\ voltage\ Measured}} \quad \textbf{... (2)}$$

In the case of ARDUINO:

Resolution: $2^n = 2^{10} = 1023$ (since counting begins with 0) ; n: number of bits
System voltage: 5V

Equation (2) will lead to:

$$\frac{\mathbf{1023}}{\mathbf{5}} = \frac{\boldsymbol{ADC\ reading}}{\boldsymbol{Analog\ voltage\ Measured}} \quad \textbf{..... (3)}$$

Of course, a potentiometer is used to control the analog voltage. So, we can get different values of ADC reading.

Figure (17) shows the relation between voltage and analog value

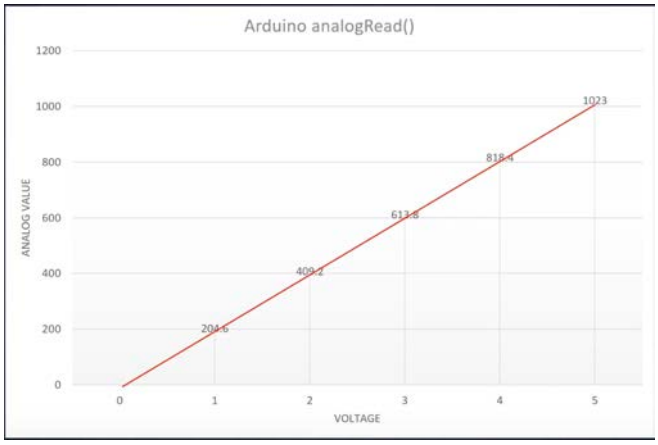


Figure 17: relation between voltage and analogread value

To calculate the analogwrite value we must write a number between 0 and 255, as equation (4) shows:

$$Write\ value = \frac{255}{1023} * Read\ value \quad \text{..... (4)}$$

Now, to modulate the LED through PWM using ARDUINO, first thing we need to keep in mind that the driver circuit of DK114N-1 is configured in the grounded common anode topology. This means, 5 V (high) represents Off and 0 V (low) represents On.

The signal cable has 8 pins illustrated in the following table:

Pin	Description
Pin 1	GND
Pin 2	Analog ADJ
Pin 3	POT
Pin 4	Enable
Pin 5	PWM
Pin 6	Vinx
Pin 7	Thermistor
Pin 8	Thermistor

Table 2: LED signal pins

To control the LED's peak current via the on-board potentiometer, analog ADJ and POT pins should be shorted. And we connect both the output (pin9) and the ground of the ARDUINO (pin 9) across Pin 5 (PWM) and Pin 1 (GND) of signal cable.

The first step done, was to design the circuit and test it using a simulation program (an LCD is used to show the voltage value and the LED on/off period to the user), figure (18).

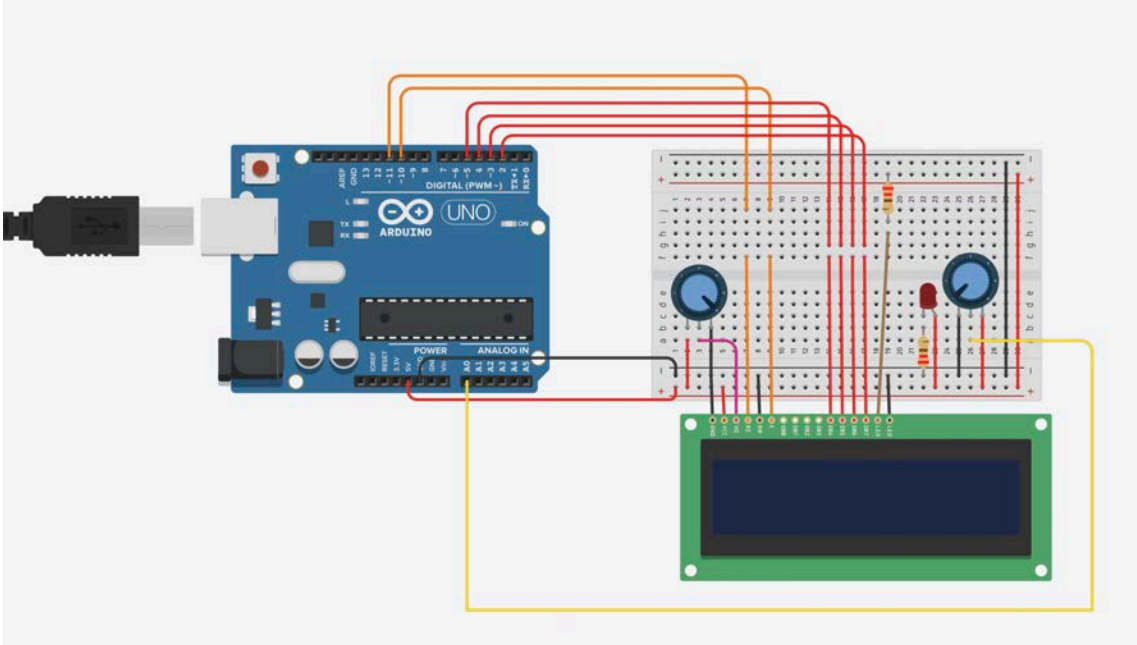
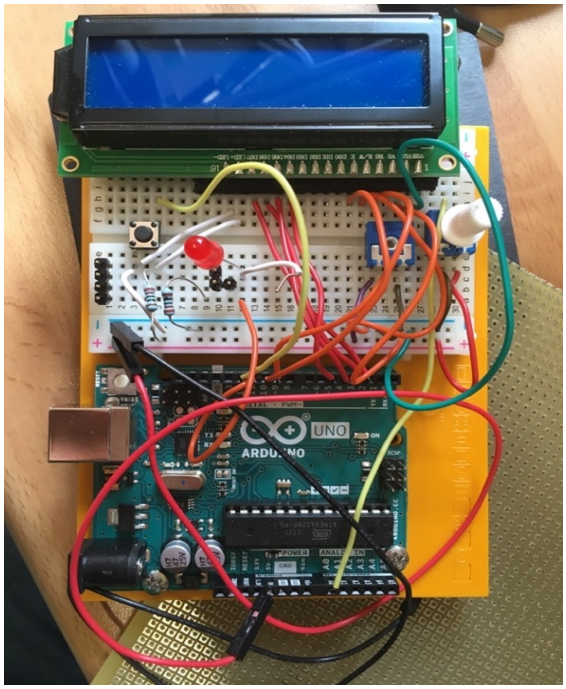


Figure 18: schematic diagram of the arduino circuit

Then, I mounted a prototype circuit using a commercial LED and prototype board.

later, I mounted the ARDUINO on a circuit board specially designed to fit in the front side of the microscope. (Fig. 19).

[A]



[B]

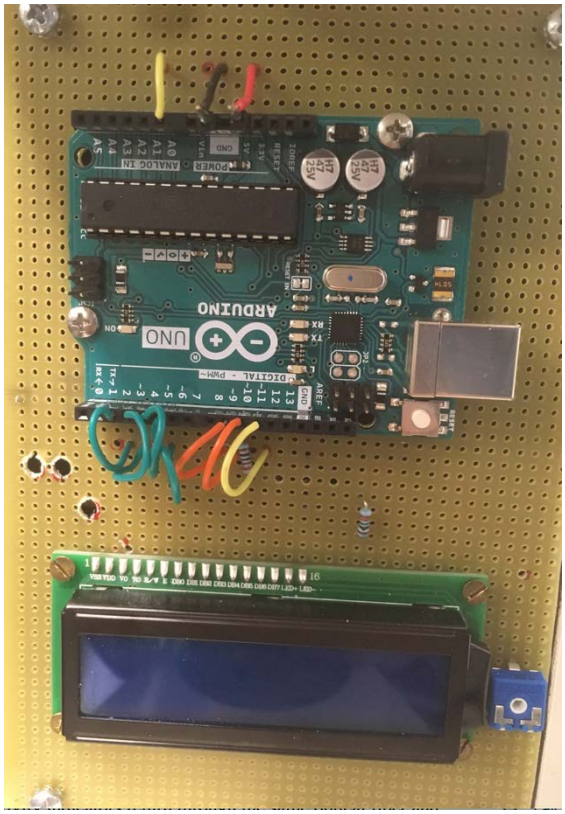


Figure 19: (A) Prototype circuit. (B) Final circuit with welded wires

I replaced the potentiometer used in the prototype circuit (10 kohm) with one of a similar value but with much more sensitivity and rotational. And I took advantage of an already existed hole on the right side of the microscope. I built two metal circles with an open hole that fits perfectly with the shaft of the potentiometer in order to fix it there (Fig. 20). In this way, the brightness of the LED can be controlled in an easy and flexible way.

[A]

[B]

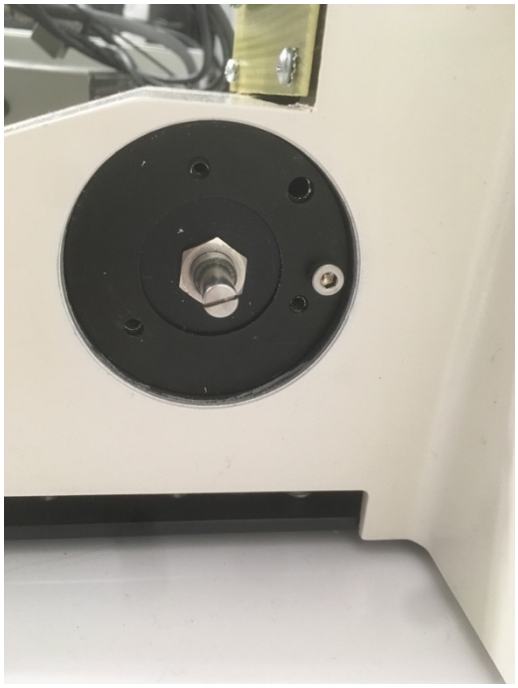


Figure 20: (A)10 kohm rotational potentiometer. (B) potentiometer rotational shaft on the right side of the device

Finally, I tested the circuit with the DK-114N LED keeping in mind that exceeding 1023 on the PWM pin may cause permanent damage to the driver board (in our case 0 V since it represents the high state). For this reason, I sat a threshold at 0.5 V. Once the voltage exceeds this value, the LED will receive a voltage of 5 V (Low state) for 10 seconds giving a time to lower the voltage again above the threshold.

The following images shows on and off states of the LED (Fig. 21).

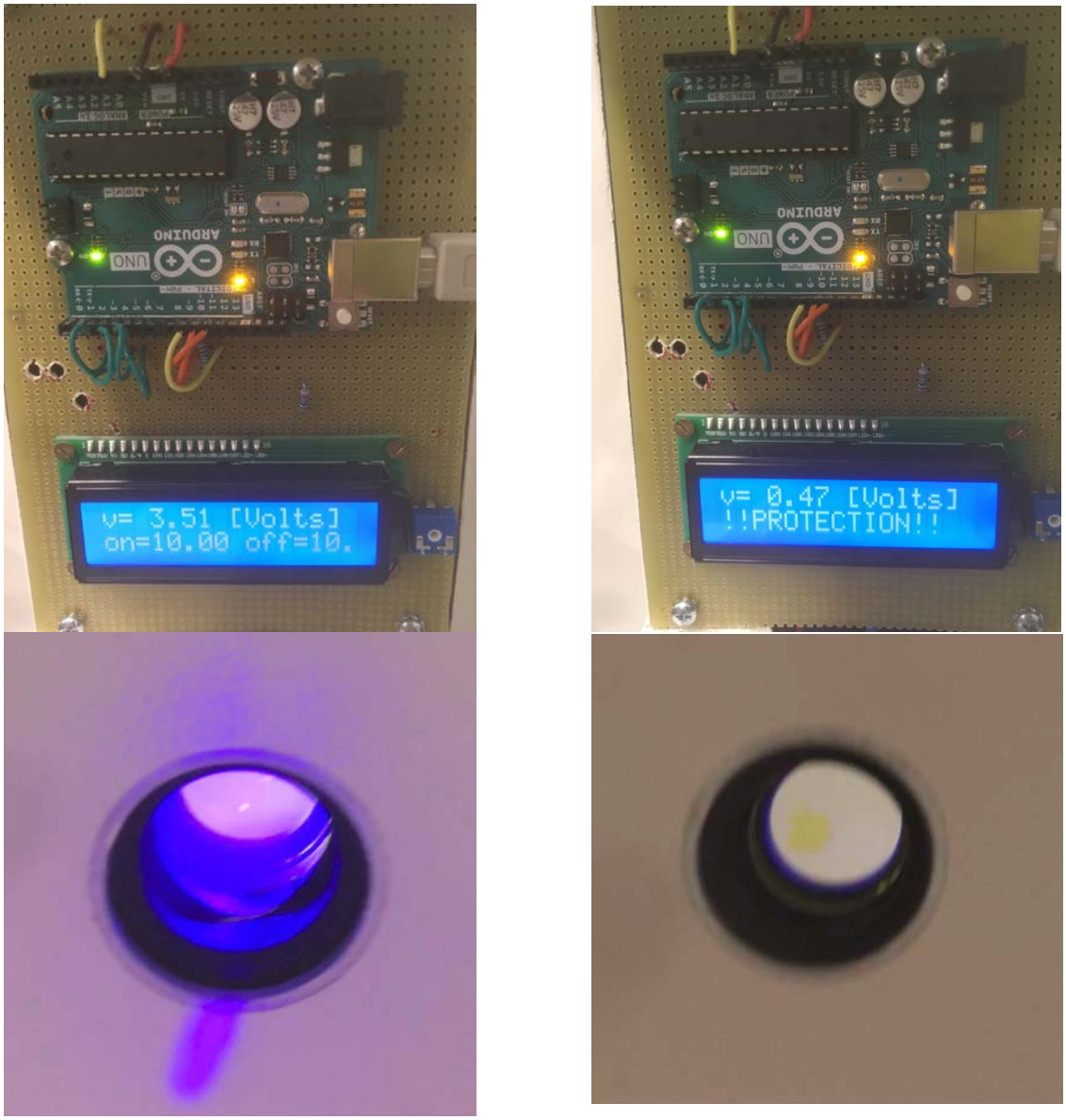


Figure 21: LED control with Arduino. On state (left), off state (right)

2.1.4. Excitation and emission filters

"Optical fluorescence occurs when a molecule absorbs light at wavelengths within its absorption band, and then nearly instantaneously emits light at longer wavelengths within its emission band" [16]. For studying the activity of certain cells and other biological molecules, fluorophores (strongly fluorescing molecules are attached to them). These fluorophores have specific range for both excitation and emission. So, before purchasing the filters we need to know our objective of the study and which fluorophore dye we are going to use.

In this project, since we are interested in studying the activity of calcium in neurons, the fluorophore we injected the animal with was eGFP (Enhanced Green Fluorescent Protein), which has the following characteristics.

GFP variant	Excitation max (nm)	Emission max (nm)
eGFP	488	509

Table 3:Excitation and emission data of eGFP

The optical spectrum of our LED is illustrated in the following figure (22)

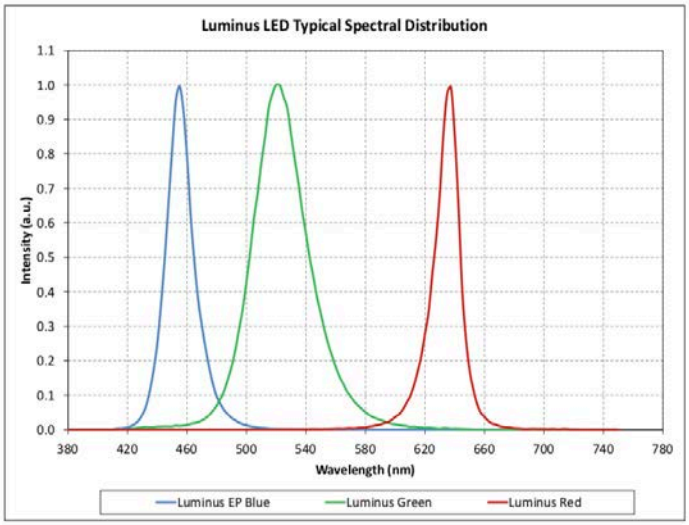


Figure 22: Luminos LEDs spectral wavelenghts

one drawback of the Luminus EP blue LED is its narrow range as well as its weak intensity near 488 nm (the excitation wavelength of eGFP). Therefore, the LED should be turned on with its almost full brightness in order to excite the fluorophore.

Giving the previous information, the components of the filter cube were chosen as the following:

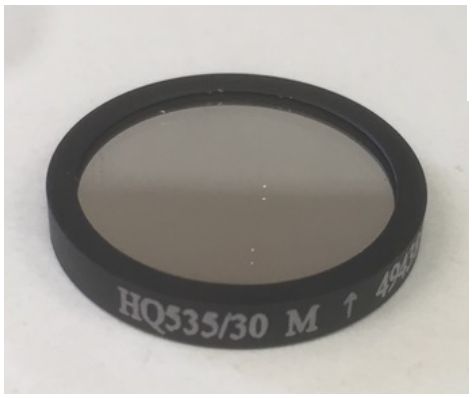
- Excitation filter (450-490 nm)

This excitation filter is compatible with the device blue LED. Also, its 40 nm bandwidth allows it to pass different intensities of the LED light.

[A]

- Emission filter (520-550 nm):

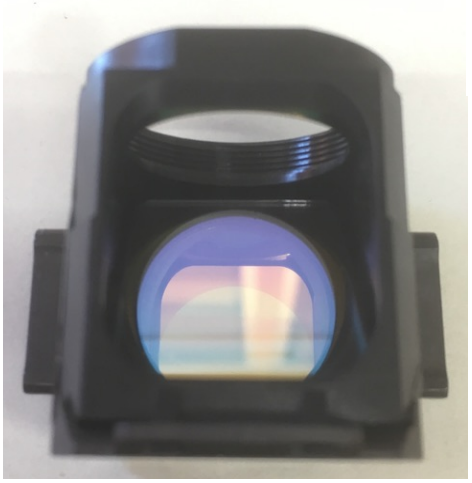
The fluorophore has a maximum emission wavelength of almost 510 nm. However, its spectral range can reach until 530 nm. With this emission filter we can detect the emitted signal perfectly.



[B]

- Dichroic mirror: (FT 515 nm):

This dichroic mirror will reflect light with wavelength less than 515 nm by 45 degrees leading it to reach the aspherical lens before reaching the optical fiber. It also, will transmit the light emitted by the fluorophore to the photodetector.



[C]

Figure 23: (A) excitation filter. (B) emission filter. (C)Dichroic mirror

2.1.5. Aspherical lens

"An aspheric lens is a lens whose surface profiles are not portions of a sphere or cylinder. The asphere's more complex surface profile can reduce or eliminate spherical aberration and also reduce other optical aberrations such as astigmatism, compared to a simple lens" [17] (Fig. 24)

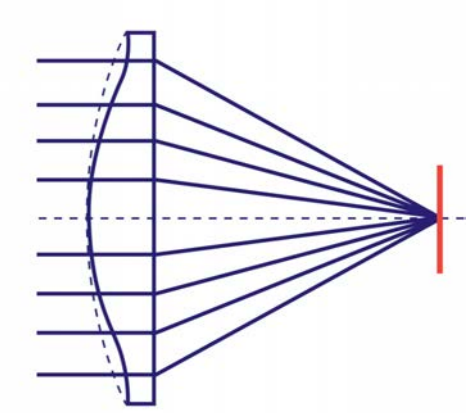


Figure 24: principle of the aspheric lens

To focus the light at the entrance of the optical fiber, an aspheric lens with a focal length of 20.1 mm and a diameter of 25 mm was purchased (Fig. 33) and placed inside a cylindrical cube (with an internal diameter of 25 mm) at about 20 mm distance from the optical fiber (Fig 25).

[A]

[B]

Figure 25: (A) Aspheric Condenser Lens, Ø25 mm, f=20.1 mm, NA=0.60, Uncoated. (B) cylindrical tube where the aspheric lens is place.

2.1.6. Optical fiber

There are two main types of optical fibers, single-mode fiber and multimode fiber [18].

Single-mode fiber is normally used when the light has to travel a long distance since its glass fiber core has a small diameter and therefore the attenuation of the signal strength is minimized and a more direct route for the light is allowed. Usually, laser is used as a light source in this type.

On the other hand, multimode fiber is used with applications that require the light to travel a short distance since its core diameter is relatively large causing the light to bounce and reflect more along its path and allowing multiple light pulses to travel through the cable at the same time. Usually, LEDs are used as a light source in this type.

Another important feature that affects how an optical fiber acts is its numerical aperture (NA) which is by definition: a dimensionless number that describes the acceptance of cone of an objective and hence its resolution and light gathering ability. [19]

The larger the core diameter and the NA, the bigger the lesion, but the larger the illumination volume. Normally, optical fiber between 200-400 µm are used in photometry systems depending on which area in the animal's brain we are dealing with (Fig. 26).

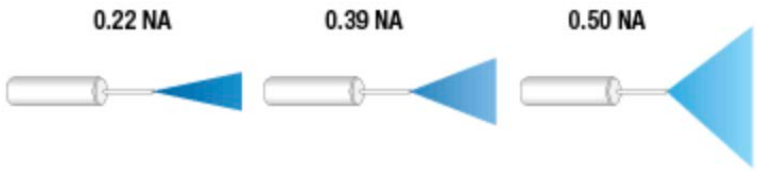


Figure 26: areas of optical fiber projected light using different NA [20]

In this project, since our light source is a LED, a multimode optical fiber (400 µm core diameter; 0.39 NA) [21] was purchased (Fig. 27) in addition to Externally threaded Fiber adapter and Ceramic Split Mating Sleeve for Ø1.25 mm (LC/PC) Ferrules.

Figure 27: M99L01 - Ø400 µm Core, 0.39 NA FC/PC to Ø1.25 mm Ferrule Patch Cable, 1 m Long

2.1.7. On/Off switch

To turn the LED On and Off, an On/Off switch with a LED is added. Before doing the electrical connections, the switch was tested with a multimeter to find the common point before soldering the wires and placing it on the right side of the microscope (Fig. 28)

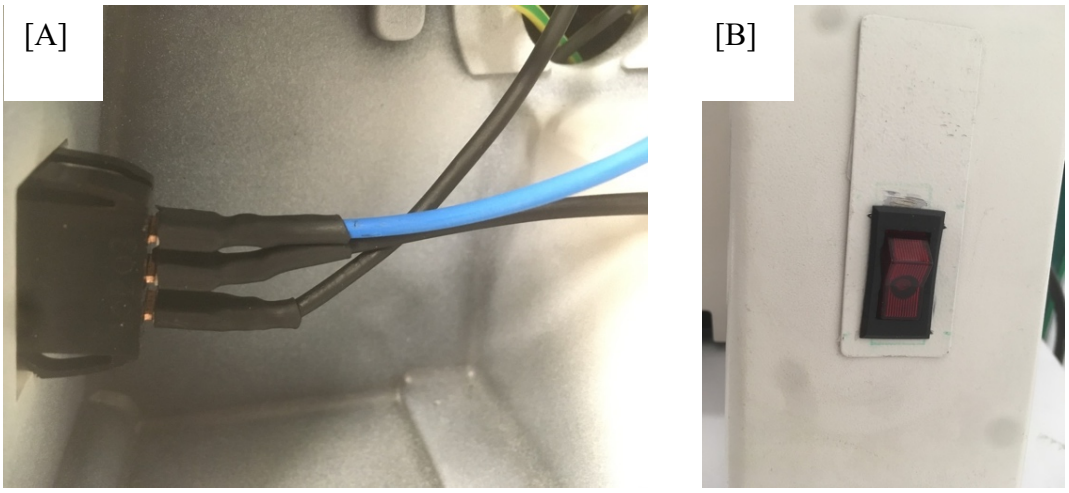


Figure 28: (A)on/off switch electrical connection. (B) on/off switch position on the right side of the device

Also, a place for an AC power connector was designed in the back side of the microscope (Fig. 29). And the wire connection between the connector, converter, and on/off switch were hidden inside the microscope.

Figure 29: AC power connector place on the back side of the device

Figure (30) shows a schematic diagram for the electrical connections.

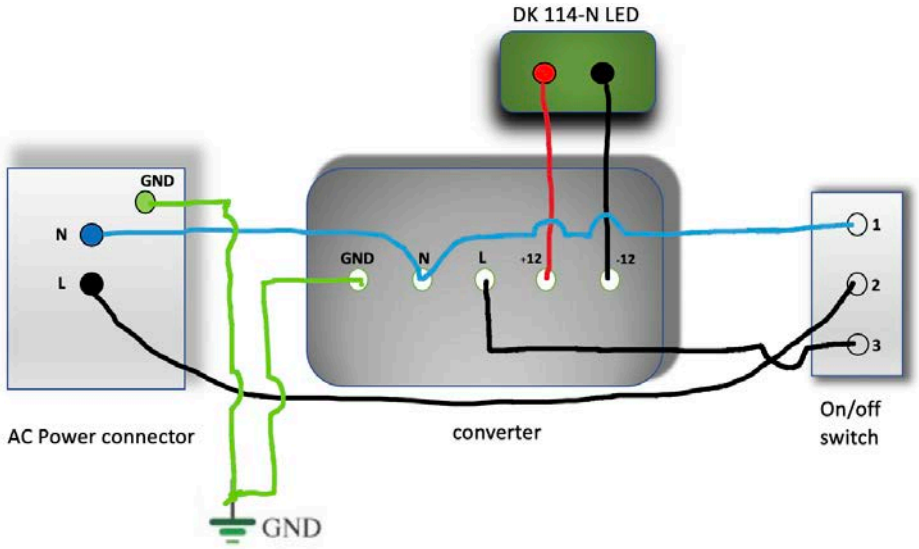


Figure 30: schematic diagram for the electrical connections

2.1.8. Photomultiplier

Photomultiplier tubes (PMTs) are generally used as the photodetectors because of their fast response, high signal to noise ratio (SNR), and above all their high gain. These characteristics made them extremely sensitive to light.

PMTs are used when light flux is extremely low. A high light flux may harm the photomultiplier.

The basic principle behind this device is illustrated in figure (31).

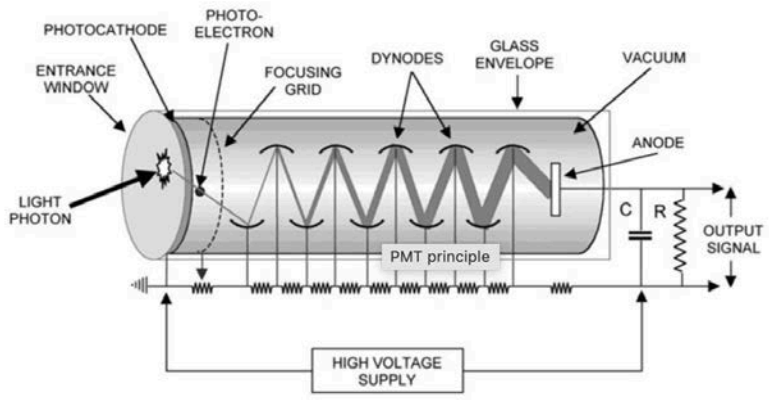


Figure 31: principle of photomultiplier [22]

For this project, (H7422-40, Hamamatsu, Japan) photosensor was mounted from scratch. Its main advantage is offering high detectivity in a spectral range between 300-720 nm.

In order to connect it with the rest of the system, the top side of the filters cube turret was modified in order to fit precisely a metal piece that could be connected to the photo sensor through a C-mount. Figure (32) shows these modifications.

Figure 32: filters cube turret top side before modification (left) and after modification (right)

A precise fitting of the photomultiplier to the equipment is necessary, so we assure that there would not be a leakage of light that might harm the photosensor (Fig. 33).

Figure 33: direct connection of the photosensor to the device through C-mount

The photosensor came with a power unit (Fig. 34) and a heat fan. The power unit (C8137-02) is responsible for providing AC input for the module, the heat fan and the peltier element (provides a better signal to noise ratio since by reducing thermal noise).

Figure 34: power supply unit

An important feature of the power unit is the protection circuit, which stops high voltage supplying to the photomultiplier whenever an excessive light reaches the photosensor.

2.1.1. Current amplifier

The output signal of the photomultiplier is relatively small (we are talking about detecting of photons), a current amplifier is needed in order to magnify the signal before sending it to the ADC and monitoring it by Lab chart software.

A low noise current amplifier from (Stanford research systems, California, USA) (Fig. 35) was used. It has many features that can help us to detect more precise signals. It can: control bias voltage, input offset, filter unwanted frequencies and most importantly it has a very wide range of sensitivity.

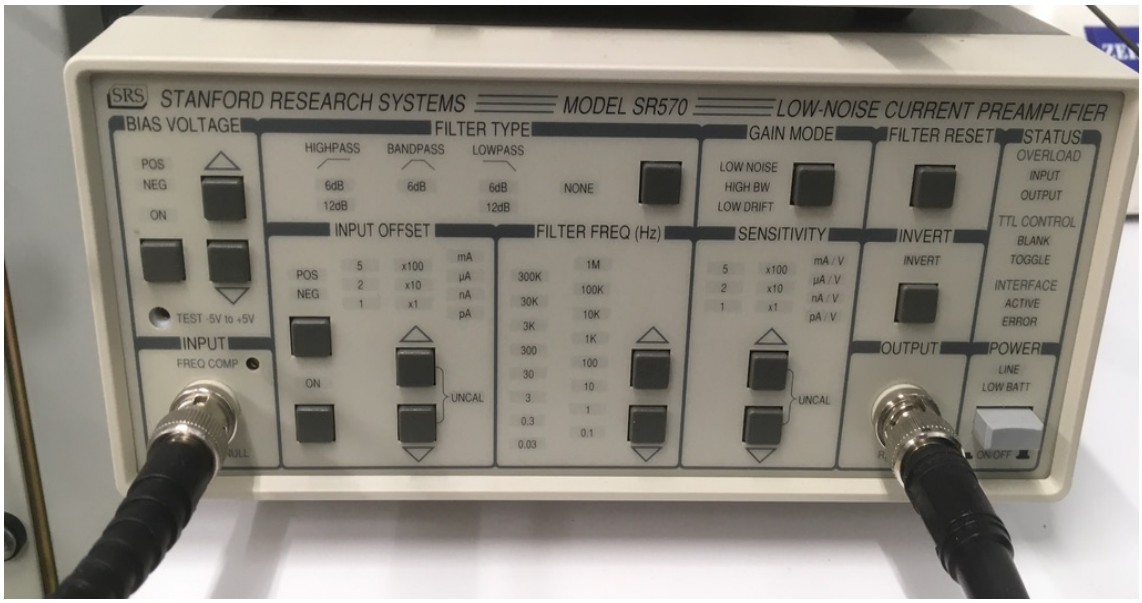


Figure 35: Current amplifier

2.1.2. Noise eliminator

When studying biological signals, a care should be taken that the signal is not affected by the different sources of noise.

The main source of noise in this project is powerline interferences, since there are a lot of electrical connections surrounding the device. Normally, to get rid of this noise (50/60 Hz depending on the country) a notch filter is used. Notch filter will overwhelm 50/60 Hz and harmonics, which is not preferable when studying certain biological signals with frequencies close to the filtered frequency and they will be distorted as well.

In this project, I used a (HUM BUG, quest scientific, Canada) noise eliminator, which has many advantages over a conventional notch filter [23].

- It does not attenuate the signal or introduce a phase error recorded signal.
- No frequency loss or DC voltage shaft
- It eliminated 50/60 Hz noise without affecting the physiological signal.

The output signal of the amplifier pass through the noise eliminator before going reaching the data acquisition system. see figure (36)

Figure 36: noise eliminator

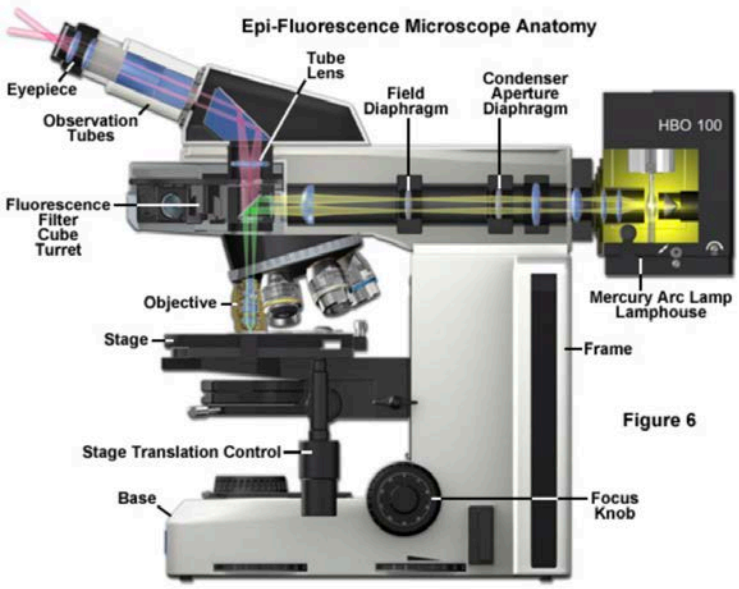
2.1.10. Virus injection

A specialized person injected 1 μL of adeno-associated virus serotype 1 (AAV1) carrying the calcium indicator GCaMP6m in a double-floxed inverted open reading frame under the control of the Synapsin promoter (AAV1-hSyn- Flex-GCaMP6m). The virus was injected bilaterally into somatosensory cortex of an anesthetize adult mouse fixed to a stereotactic frame , at coordinates -1.0 anteroposterior (AP), 3 mediolateral (ML), and 1.5 dorsoventral (DV), measured from bregma on the skull surface. Injections were performed using a glass injector pipette and a Micro-4 Injector system (World Precision Instruments, Inc). The needle was held in place for 1 min before the start of injection, injection speed was 100nL min−1, and the injection needle was raised 5min after completion of virus delivery [24].

2.2. Final system assembly

Many modifications have been made to the microscope to adjust it to fit perfectly with the different components of the photometry system. (Fig. 37) shows the anatomy of the microscope before and after these modifications.

[1]



[2]

Figure 37: (1) microscopy anatomy before modification. (2) microscope anatomy after modification, (A) LED on the back side, (B) converter inside the microscope, (C) Arduino, (D) Filter cube, (E) Aspheric lens, (F) optical fiber, (G) on/off switch, (H) photomultiplier, (I) potentiometer

In figure (38) we can see the final assembly of the system

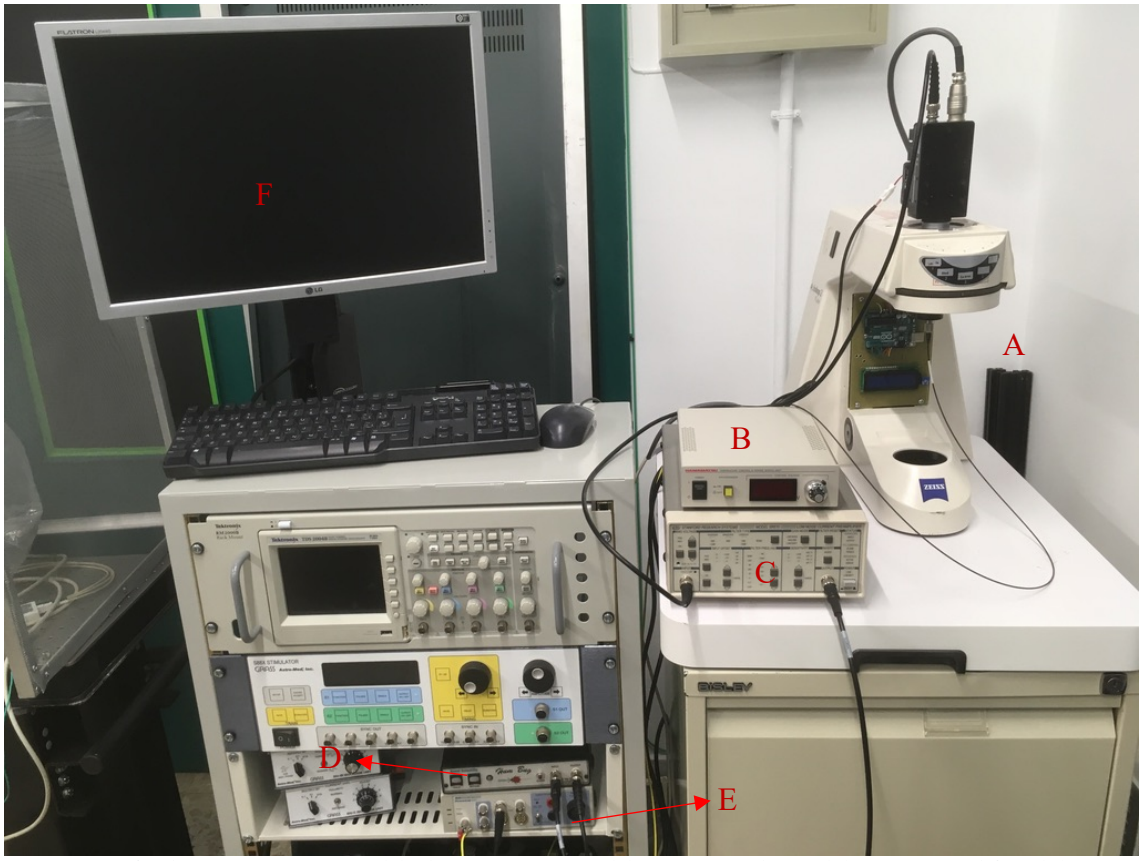


Figure 38: final system components. (A) photometry system, (B) PMT control unit, (C) current amplifier, (D) noise eliminator, (E) data acquisition system, (F) monitor

Finally, a schematic diagram of the final system is illustrated in figure (39).

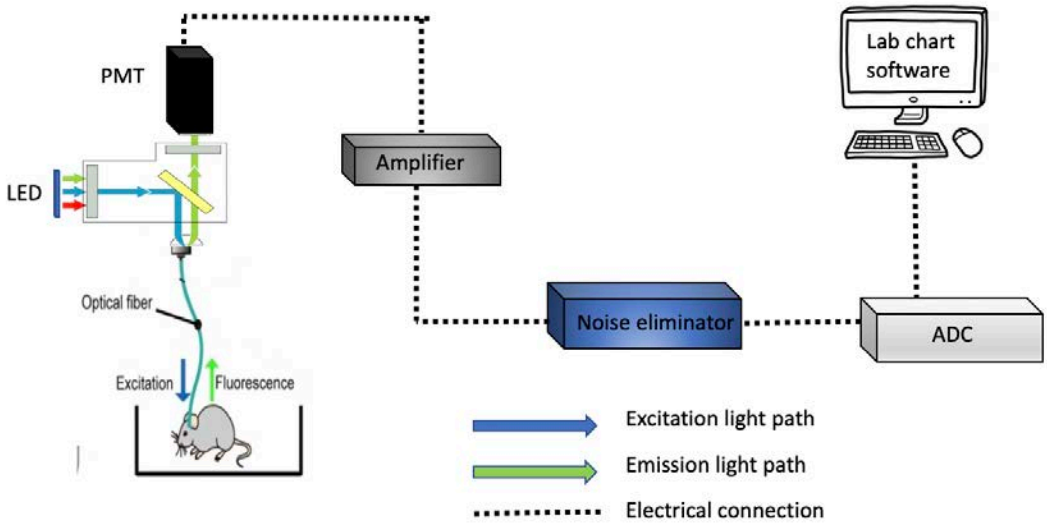


Figure 39: schematic diagram of the system final assembly

2.3. System initiating protocol:

For an appropriate initiation of the system, it is preferred to do the following step:

1) Turn off the room light.
2) Turn on the PC and connect the USB cable to Arduino.
3) Set the frequency you desire and upload the code again.
4) Make sure that the filter cube turret is placed at position 1.
5) Turn on the power of power supply of the PMT.
6) Check the upper window of the control voltage adjustment dial reads “0”
7) Wait for 3 minutes till the unit set to standby.
8) While waiting, put the slider located in front of the LED on close mode.
9) Rotate the potentiometer shaft clockwise until you read on the LCD [5V] (set the LED voltage to minimum).
10) Turn on the current amplifier, the noise eliminator and the data acquisition system.
11) Open Lab chart on the computer and set number of channels to 1channel.
12) Turn on the LED
13) Adjust the sensitivity of the photosensor by turning clockwise the control voltage shaft.
14) Turn on the photosensor switch on C8137-2
15) Connect the optical fiber to the cannula located on the animal implant.
16) Open the slider
17) Increase the intensity of the light by rotating the potentiometer shaft counter clockwise.
18) Make sure that the parameters of the amplifier are sat appropriately.

3.Results and Discussion:

3.1. testing the system with LED:

Before testing the system with a live animal, a test for the system ability to detect the light and respond in an appropriate way had to be performed.

For this reason, a green commercial LED was used to demonstrate the ability of the optical fiber to pass the emission light. figure (40)

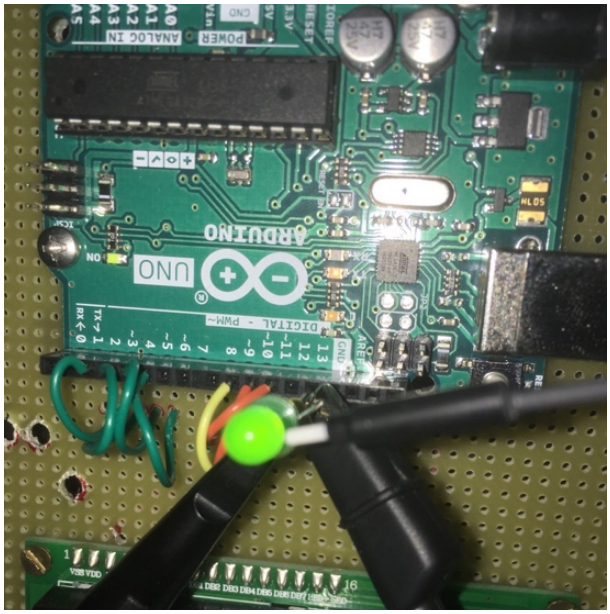


Figure 40: optical fiber detecting the light of a Green LED

Also, a different filter cube with the following characteristic was used:

Filter cube	Excitation	Dichroic mirror	Emission
Filter set 05	BP 395-440	FT 460	LP 470

Table 4: characteristics of the filter cube used in testing the system with a green commercial LED

By using low pass emission filter, we can study the effects of different parameters of the system without being afraid that the brightness of the LED is out of the emission filter spectral range.

The green LED blinking time was set to one second on and two seconds off. The brightness of the LED was at first at its maximum (directly connected to pin 13 which has an internal resistor for LED protection).

Before performing any experiment, the lights of the room are switched off so the optical fiber do not detect any light from the surrounding environment.

a- Effect of photomultiplier control voltage:

To see if the photomultiplier is working properly, both the intensity of the LED and the sensitivity of the preamplifier (maximum 1*1 pA/V) were fixed, and the detected signal was recorded with different control voltage of the photomultiplier.

First, before tuning the LED on and off, the control voltage it was sat to 0.029 (maximum value that could be reach before saturation happens). The system had a DC offset of almost (-0.2 Volts). (Fig. 41)

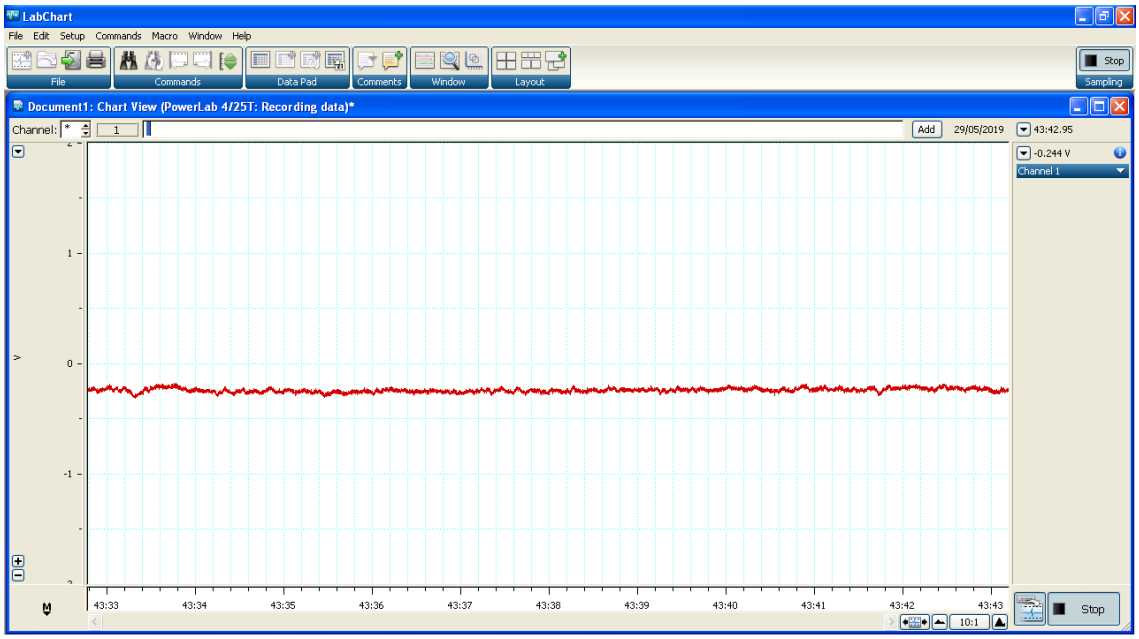


Figure 41: DC offset voltage, the LED is switched off

Then, the LED started blinking. The voltage value was almost -0.7 V (Fig. 42).

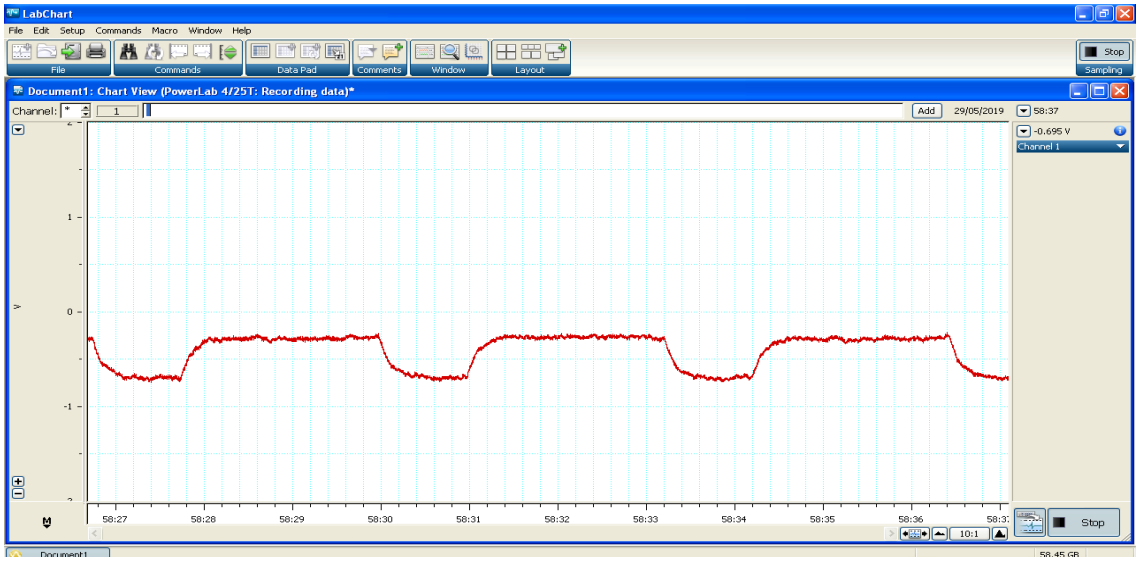


Figure 42: recorded signal with maximum: brightness, amplifier sensitivity and control voltage

Increasing the control voltage to 0.03, we reach saturation (Fig. 43).

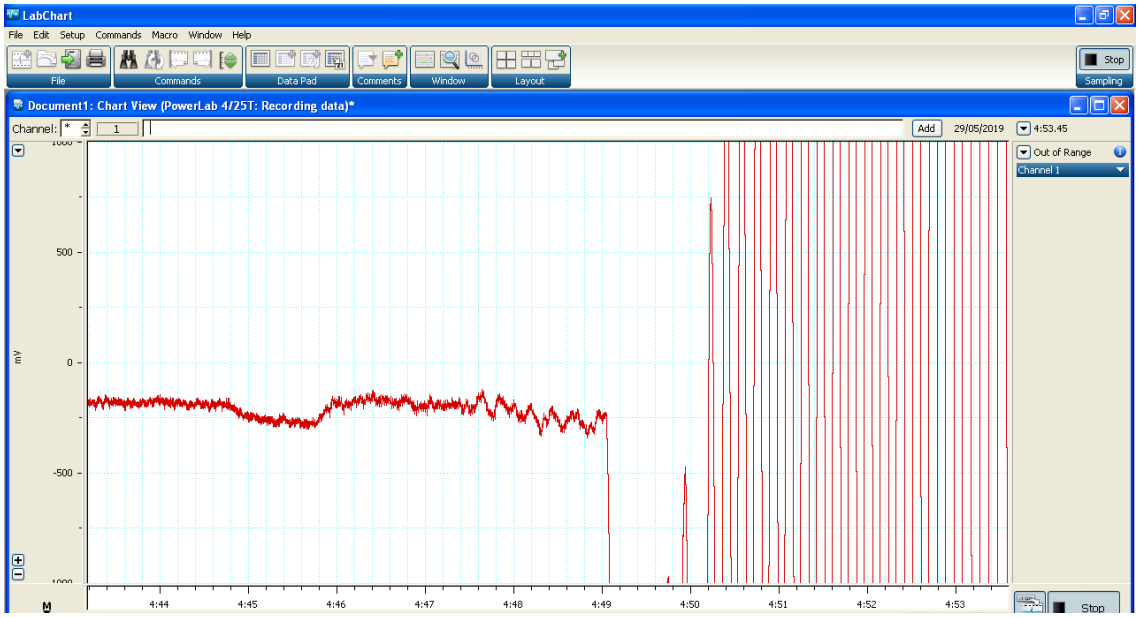


Figure 43: signal recorded when control voltage of the PMT increased to 0.03

Decreasing the control voltage to 0.01, we notice that both DC offset and the recorded signal have decreased (Fig. 44).

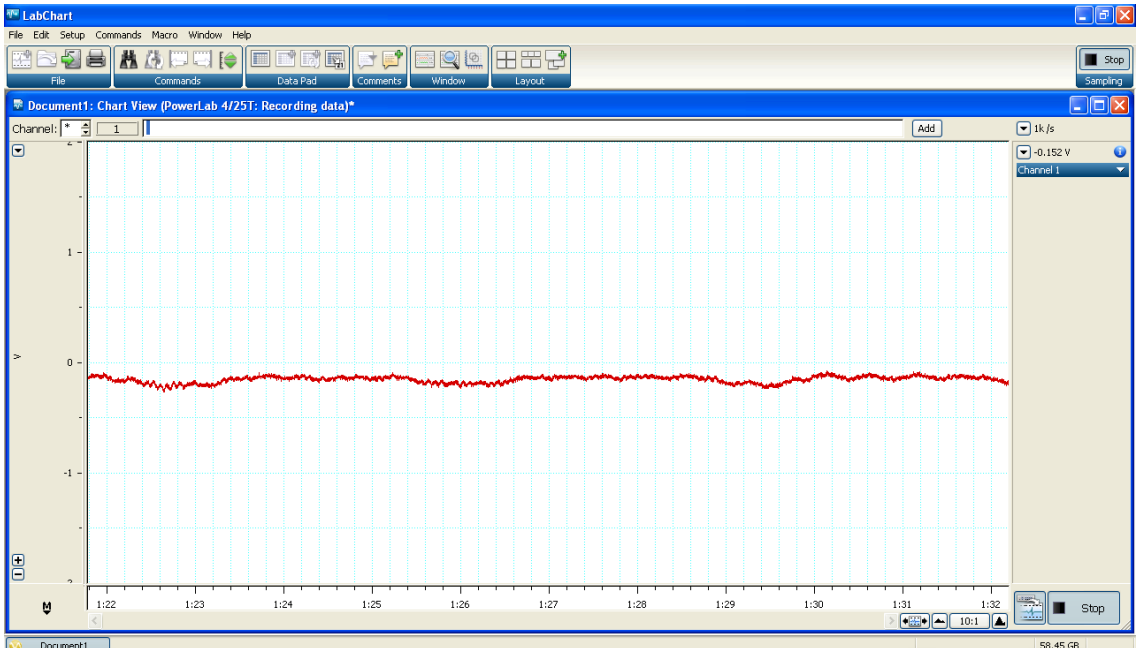


Figure 44: recorded signal with maximum brightness and amplifier sensitivity, but with minimum control voltage

b- Effect of LED intensity (brightness)

This time, both the control voltage and preamplifier sensitivity were fixed to study the effect of the LED brightness on the recorded signal (Fig. 45).

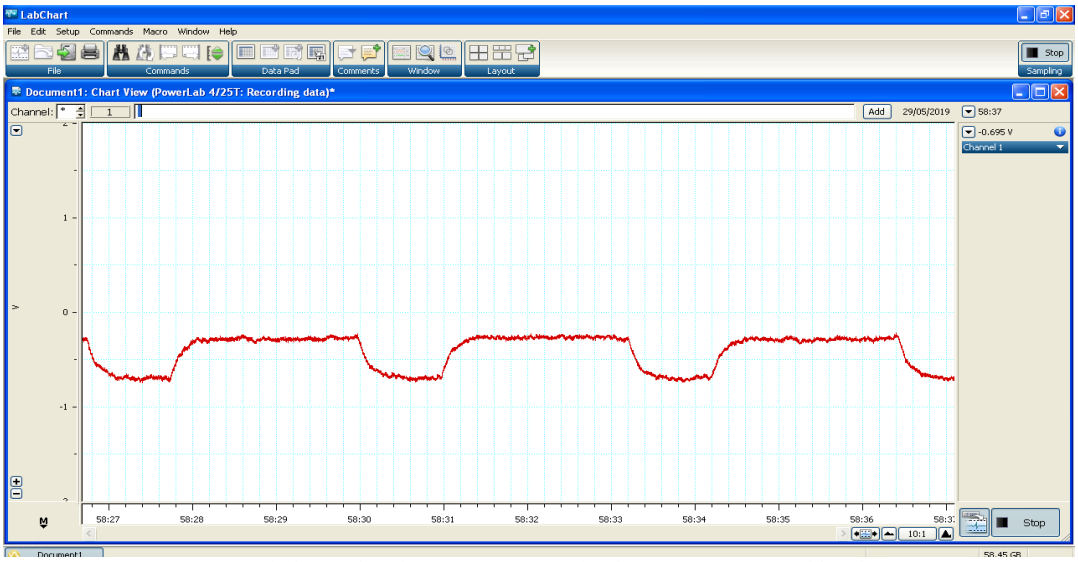


Figure 45: recorded signal with maximum control voltage, sensitivity and brightness

Dimming the light with 1Kohm resistor, there is a notable decreasing in the recorded signal (Fig. 46).

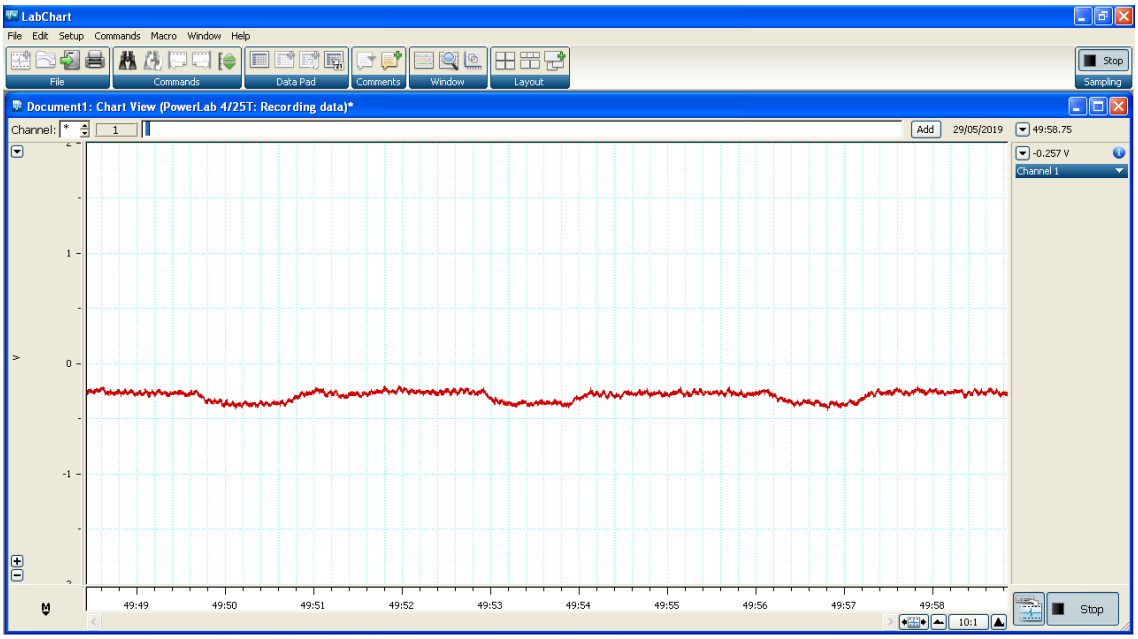


Figure 46: recorded signal with máximum control voltage and sensetivity, but with attenuated LED signal

There would be no detected signal if dim the light using a 10 Kohm resister (Fig. 47).

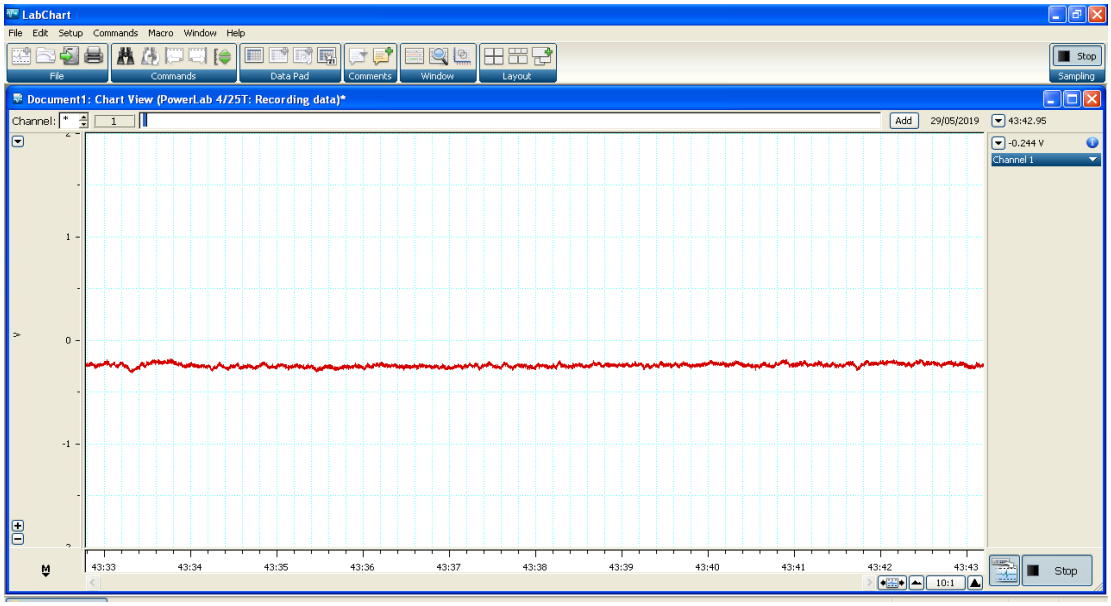


Figure 47: recorded signal with maximum control voltage and sensitivity, but with very attenuated dim brightness

c- Effect of noise eliminator:

In all previous measurements, the output of the preamplifier was connected to the noise eliminator before and then to the data acquisition system. To see the effect of the noise eliminator, a direct connection between preamplifier and the data acquisition system has been implemented.

The voltage value of the recorded signal was not affected, but the signal was accompanied with an obvious 50Hz noise coming from the surrounding electrical connections (Fig. 48).

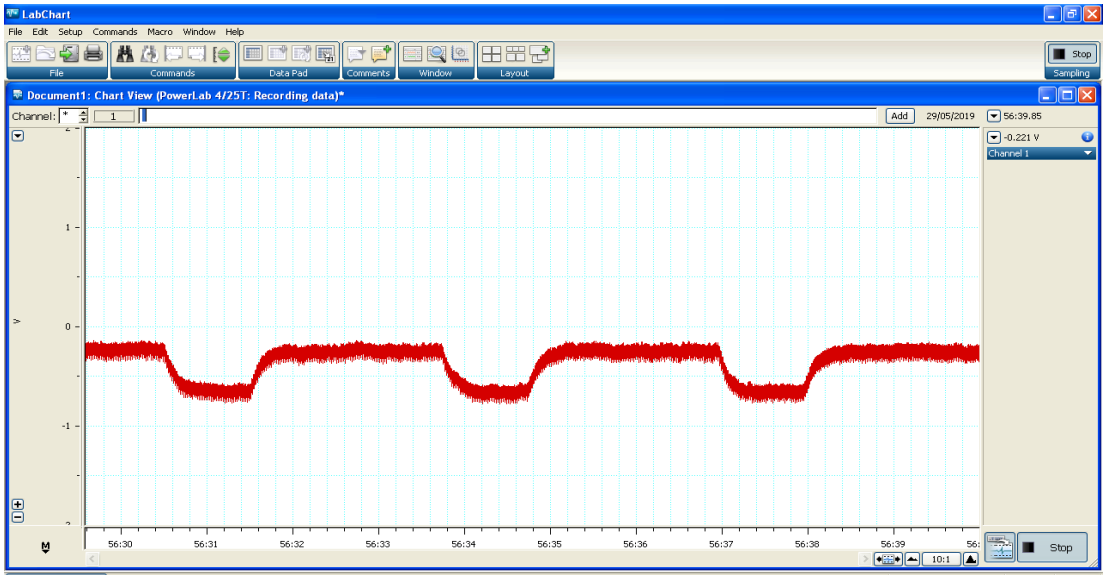


Figure 48: recorded signal with maximum: brightness, amplifier sensitivity and control voltage without using the noise eliminator

d- Effects of the preamplifier sensitivity

Now, to see the rule that the sensitivity of the preamplifier plays in the recorded signal, both control voltage and brightness of the LED were fixed maximum values while changing the preamplifier sensitivity. The signal was recorded using multiple the following sensitivities:

- 1*1 pA/V (maximum) (Fig. 49).

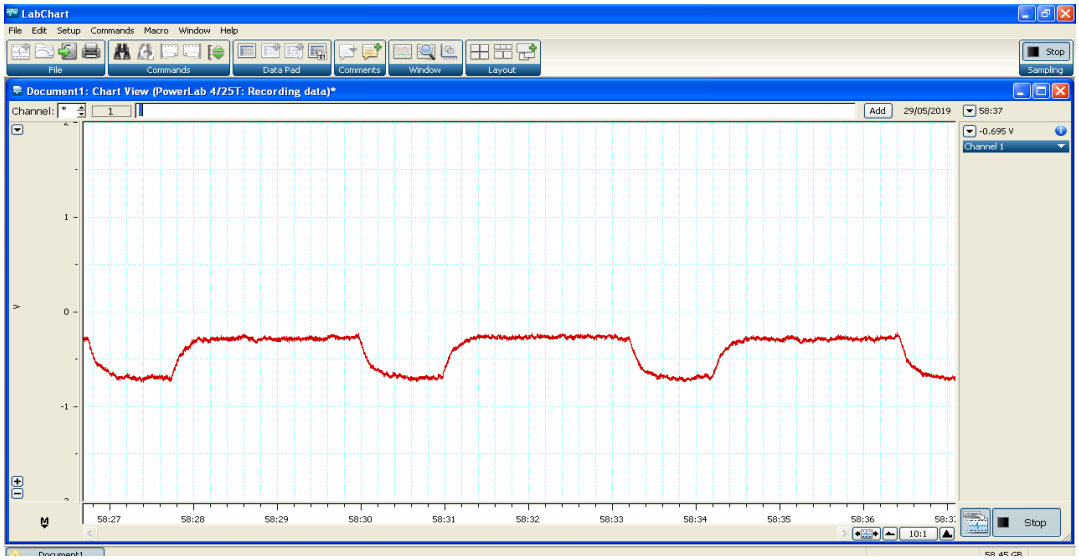


Figure 49: recorded signal with maximum: brightness, amplifier sensitivity and control voltage

- 2*1 pA/V: an obvious decreasing in the recorded signal voltage can be noticed (Fig. 50).

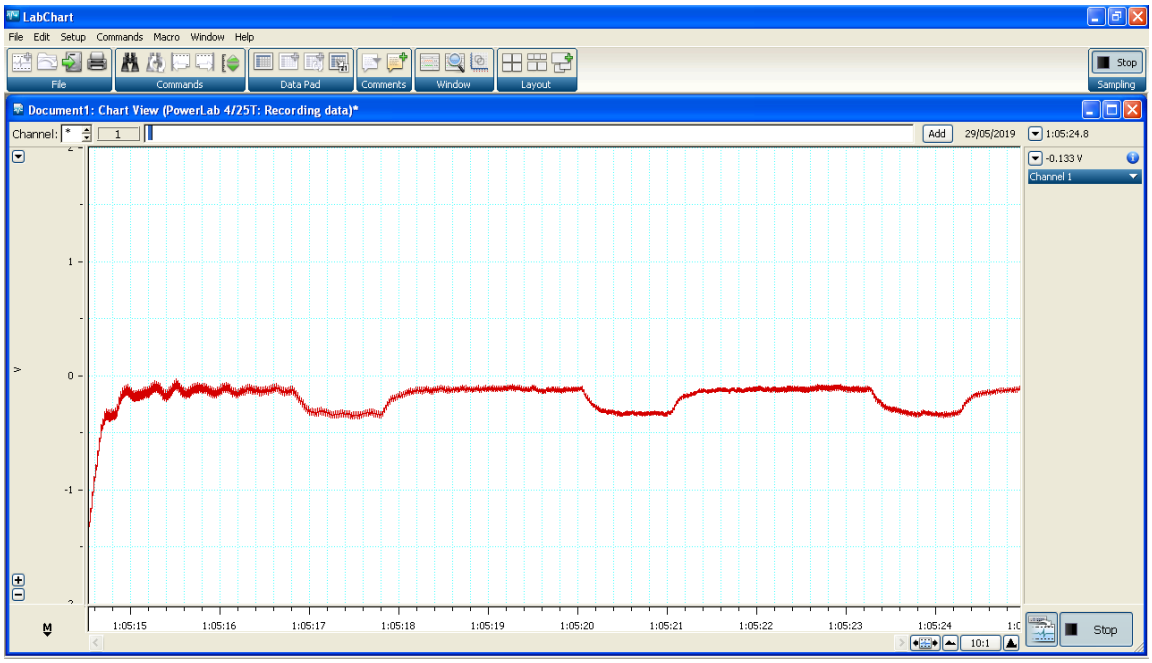


*Figure 50: recorded signal with maximum: brightness control voltage, but with amplifier sensitivity decreased to 2*1 pA/V*

This decreasing in the recorded signal voltage will continue as long as the sensitivity of the preamplifier is decreasing.

- 5*1 pA/v (Fig. 51).

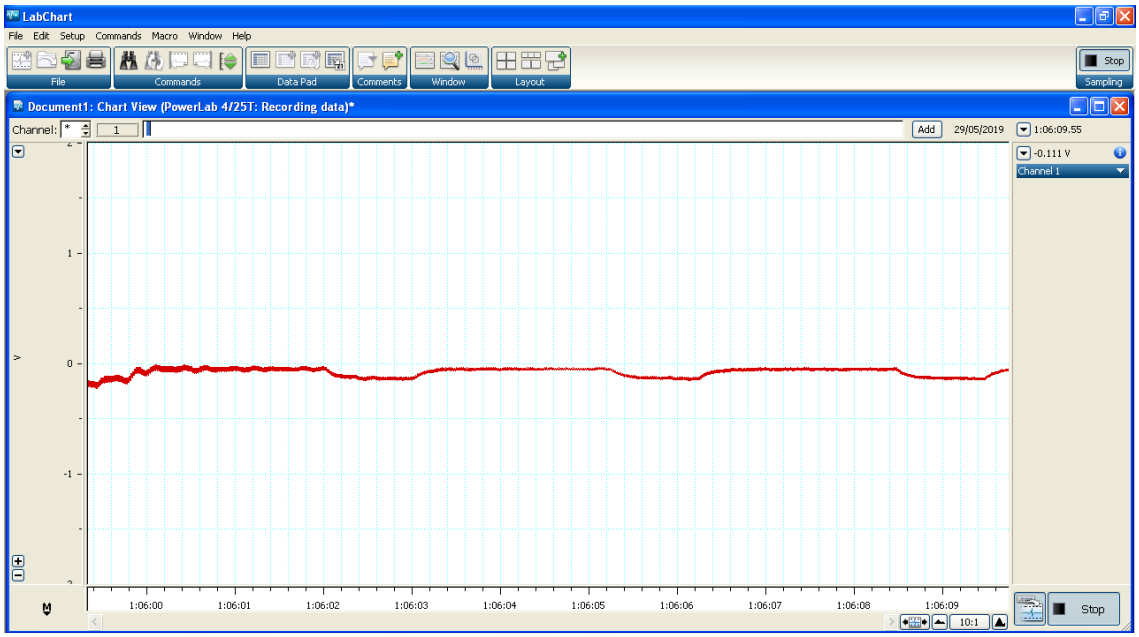


*Figure 51: recorded signal with maximum: brightness control voltage, but with amplifier sensitivity decreased to 5*1 pA/V*

- 1*10 pa/v (Fig. 52).

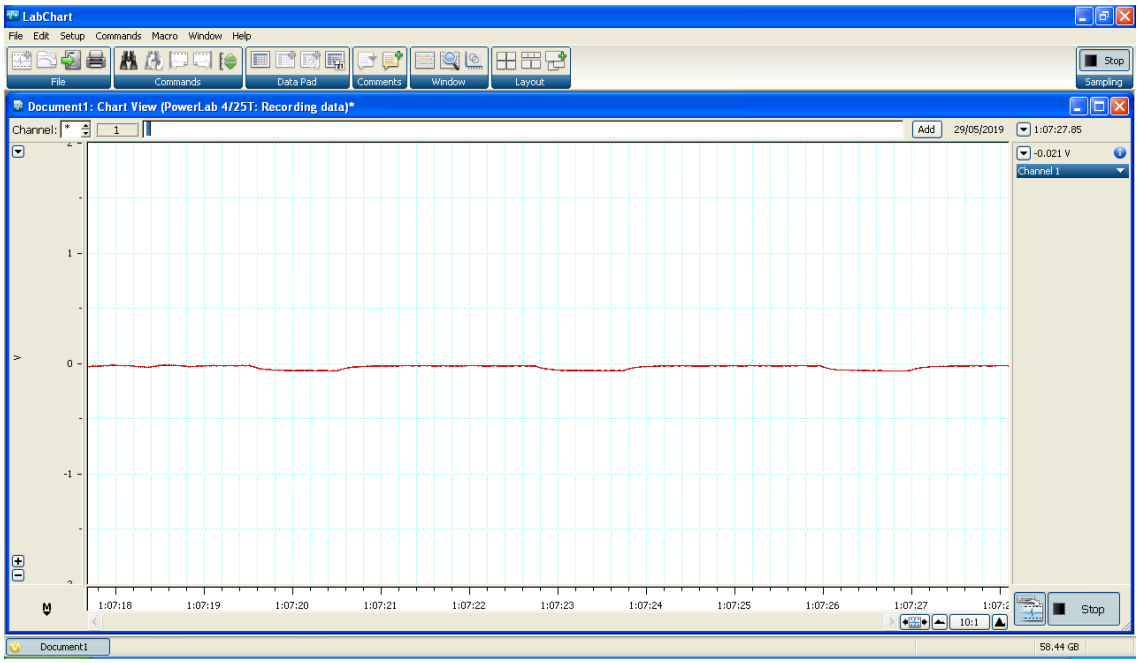


*Figure 52: recorded signal with maximum: brightness control voltage, but with amplifier sensitivity decreased to 10*1 pA/V*

e- Effect of the filter cube:

Setting the values of the LED brightness, preamplifier sensitivity, and PMT control voltage while changing the filter cube to the one designed to be used with the animal, we get a result only with a DC component (coming from the PMT). this indicates that the green LED at its maximum brightness has a wavelength not included between 520-550 nm (Fig. 53).

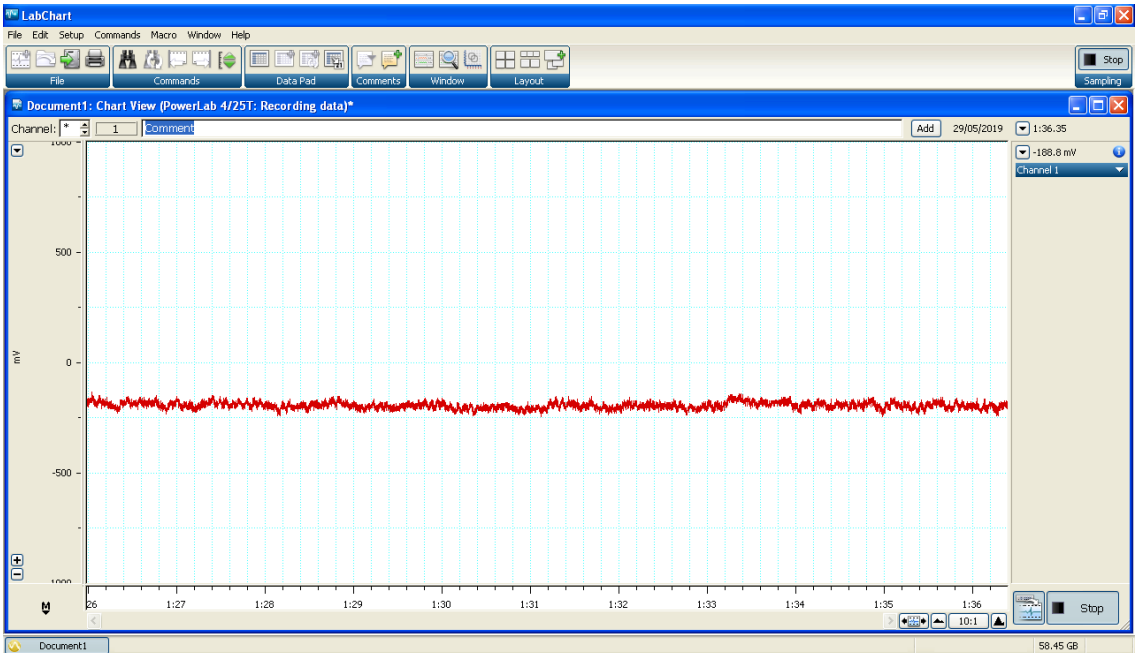


Figure 53: recorded signal when using filters set that will be used with the animal experiment

To test the PMT efficiency with time, these measurements were performed twice. In the first time, right after turning on the PMT. And in the second time 30 minutes after turning on the PMT. The results where similar which proved that the system can be working for long time without being affected.

3.2. Testing the system with animal:

After making sure that the system is working properly and responding to changes in its different parameters. An animal injected with adeno-associated virus serotype 1 (AAV1) carrying the calcium indicator GCaMP6m (Fig. 54) was used to perform a test for the system. the blinking frequency was set 10 Hz. The brightness of the LED was set to its maximum. The sensitivity of the amplifier was set to its maximum (1*1 pA/V), and the control voltage of the PMT was set to its maximum (0.029).

Figure 54: animal injected with AAV1

However, no signal was detected by the system. this might be for many reasons:
This might be for one of the following reasons:

a- Reasons related to the detecting system:
   - The excitation light wavelength might be not high enough to excite the fluorophore of injected detector.
   - The fluorophore has been excited and emitted a light with a wavelength less than 515 nm which will not be transmitted by the dichroic mirror to the PMT.

b- Reasons related to the animal:
   - The virus might not be injected properly.
   - The cannula might not be placed properly.
   - The calcium indicators might have lost their ability to respond to excitation light.

At the end, detecting calcium signals with a fluorescence photometry is very complex and needs many experiments to be performed before being able to get a result and record it. This take a lot of time, because you need a permission for injecting the animal with the virus and you have to wait for 1-2 weeks until the virus disturbs and reaches different brain regions of interest.

4. Conclusion:

4.1. current states of the system:

A full assembly of the system has been done and all the necessary connections have been implemented. An Arduino program for modifying both PWM frequency and blinking time by the user has been created. A potentiometer with a shaft placed on the side of the microscope to allow easy control of the LED brightness has been implemented.

In terms of system capabilities, it has been proved that the photomultiplier is able to detect emission light and amplify it before sending it to the data acquisition system. However, the system did not work when the optical fiber was connected the animal brain, this might be for many reasons that were discussed previously

4.2. Next steps

First step in future work is figure out the reason why the system did not work with the live animal by doing more experiments. Many approaches can be taken, we might replace the LED with one of a higher wavelength range, by this way we can assure that we are reaching the excitation wavelength of the eGFP, or we might use the system for studying another signals than calcium, whom can be encoded with fluorophores that have wavelengths closer to the Luminos blue LED spectral range.

Another step that can be done is to connect the ARDUINO pin used to control the signal of the LED to a frequency counter, so the user can see the frequency of the PWM pin on an LCD.

5. PROJECT COST:

The budget of this project includes the cost of system components, the cost of technical equipment, human resources cost.

a. Cost of system components:

These costs include all the material needed for the set-up of the fluorescence photometry system. The following table shows in detail the cost of each single element of the system. Some of the elements were already in the lab and their costs are not included.

system component	Cost (€)
LED – LED kit DK114N-1 (Luminos)	482,50 €
Excitation filter- HQ 470/40	227 €
Emission filter- HQ 535/30	227 €
Dichroic mirror- FT 515 nm	209 €
Aspheric lens - ACL2520U - Aspheric Condenser Lens, Ø25 mm, f=20.1 mm, NA=0.60, Uncoated	17 €
Optical fiber- M99L01 - Ø400 µm Core, 0.39 NA FC/PC to Ø1.25 mm Ferrule Patch Cable, 1 m Long	91 €
cannula	30 €
Arduino Uno	20 €
Photomultiplier *	----------
HUM BUG noise eliminator *	----------
Current amplifier *	----------
	1285.5 €

Table 5: system components associated costs

Note: components marked with * existed already in the laboratory where this project took place.

b. Technical equipment costs:

These represents the overhead costs of this project, which include the little extras paid for technical equipment used in the project.

Technical Equipment	Cost/month (€)	Months used	Total cost (€)
Lab computer	10	2	20
Digital Oscilloscope	10	2	20
Arduino software	0	4	0
LabChart software	0	2	0
			40 €

Table 6: technical equipment associated costs

c. Human resources costs:

These costs represent the salaries of the team members who worked on the project.

Human resources	Hours	Cost/ Hour (€)	Total cost (€)
Student	380	0	0
Supervisor	150	40	6000
			€ 6000

Table 7: human resources associated costs

The final cost:

Technical Equipment	Cost (€)
System components	1285.5
Technical components	40
Human resources	6000
	7.325,5 €

Table 8: total cost of the project

6. Annex:

6.1. Reference sheet

System initiating protocol

For an appropriate initiation of the system, it is preferred to do the following step:

1) Turn off the room light.
2) Turn on the PC and connect the USB cable to Arduino.
3) Set the frequency you desire and upload the code again.
4) Make sure that the filter cube turret is placed at position 1.
5) Turn on the power of power supply of the PMT.
6) Check the upper window of the control voltage adjustment dial reads “0”
7) Wait for 3 minutes till the unit set to standby.
8) While waiting, put the slider located in front of the LED on close mode.
9) Rotate the potentiometer shaft clockwise until you read on the LCD [5V] (set the LED voltage to minimum).
10) Turn on the current amplifier, the noise eliminator and the data acquisition system.
11) Open Lab chart on the computer and set number of channels to 1channel.
12) Turn on the LED.
13) Adjust the sensitivity of the photosensor by turning clockwise the control voltage shaft.

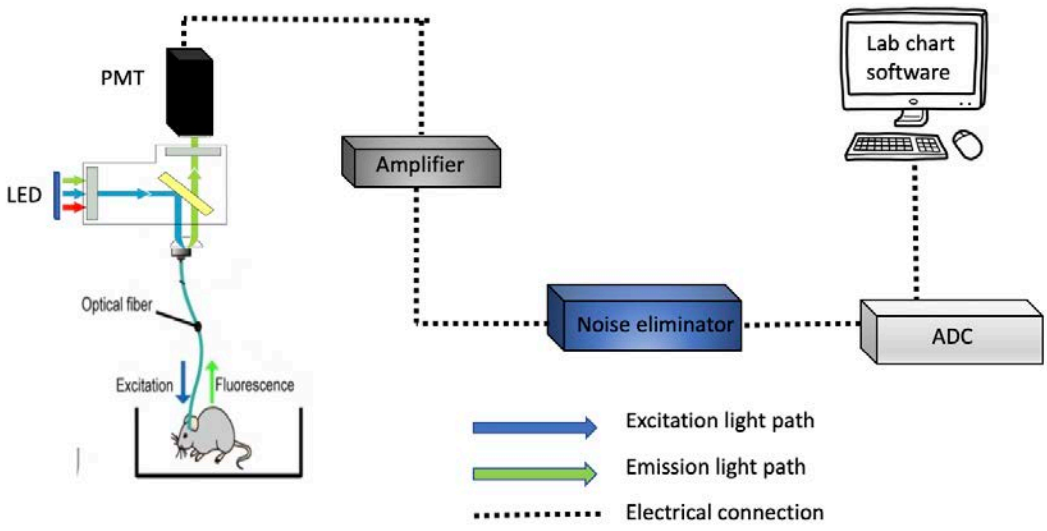
14) Turn on the photosensor switch on the PMT control unit.

15) Connect the optical fiber to the cannula located on the animal implant.

16) Open the slider

17) Increase the intensity of the light by rotating the potentiometer shaft counter clockwise.

18) Make sure that the parameters of the amplifier are sat appropriately.



6.2. Arduino code

- With PWM library:

```
//Controling the LED blinking frequency and PWM frequency

#include <PWM.h> // make sure that your IDE include this library
#include <LiquidCrystal.h>

const int switchPin = 12, rs = 11, en = 10, d4 = 5, d5 = 4, d6 = 3, d7 = 2 ; // defining the Arduino pins
LiquidCrystal lcd (rs, en, d4, d5, d6, d7);

int switchState = 0;
int prevswitchState = 0;
int reply;
int POT = A0; //declare POT as arduino input PIN A0
int LED = 9; //declare LED as Arduino output PIN 9
int readValue; // use this variable to look at the 0-5 potentiometer voltage at PIN A0
int writeValue;  // use this variable to write 0-5 V to the LED
float Voltage;

float frequencyon = 10 , frequencyoff = 10; //enter blinking frequency in Hz (on/off time)
int32_t frequency = 30; // enter the desired PWM frequenvy in Hz.


void setup()
{
Serial.begin(115200);   //ture the serial port @ 115200

InitTimersSafe(); // makes all the timers except for timer 0 available for setting different frequencies
SetPinFrequencySafe (LED, frequency);

pinMode(LED,OUTPUT);
pinMode(POT,INPUT);
pinMode(switchPin, INPUT);
lcd.begin(16, 2); // set up the LCD's number of columns and rows:
lcd.clear();

}
void loop()
{


    readValue = analogRead(POT); // read the vcalue from the potentiometer
    writeValue= (255./1023.)*readValue; // do the conversion from 1023 to 255 scale
    pwmWrite(LED, writeValue); //value: the duty cycle: between 0 (always off) and 255 (always on). Allowed data types: int
    Serial.print("analog value to Led:");
    Serial.println(writeValue);
    Voltage = (5./255.)* writeValue ;
    delay(5);
    Serial.print ("analog voltage to the LED: ");
    Serial.println (Voltage);


  if ((Voltage < 5) && (Voltage>0.5)) // Exccessing 5 V on the on the PWM pin of the LED signal cable may cause permenant damage, for this reason a threshold is sat at 0.5 (4.5 V)
   {
    lcd.clear();
    lcd.setCursor(0,0);
    lcd.print("v= "); lcd.print(Voltage); lcd.print(" [Volts]");
    lcd.setCursor(0,1);
    lcd.print ("on="); lcd.print(frequencyon); lcd.print(" off="); lcd.print(frequencyoff);
    pwmWrite(LED, writeValue);   // turn the LED on (HIGH is the voltage level)
    delay(1000/frequencyon);    // wait for X mSec ( in order to set different frequencies choose between 20, 50, 100, 200 [mSec] to set the frequency to 50, 20, 10, 5 [hz] in order.
    digitalWrite(LED, HIGH);
    delay(1000/frequencyoff); // turn the LED off by making the voltage LOWd


    }

   else  // Voltage higher than 5 or less than .5 V
    {

    lcd.clear();
    lcd.setCursor(0,0);
    lcd.print("v= "); lcd.print(Voltage); lcd.print(" [Volts]");
    lcd.setCursor(0,1);
    lcd.print("!!PROTECTION!!"); // in order to protect the LED from high control voltage
    digitalWrite (LED ,HIGH);
    delay(10000);

      }
      delay(10); // for stabilization
}
```

- Without the PWM library

```
//Controling the LED blinking frequency and PWM frequency

#include <LiquidCrystal.h>
const int switchPin = 12, rs = 11, en = 10, d4 = 5, d5 = 4, d6 = 3, d7 = 2 ; // defining the Arduino pins
LiquidCrystal lcd (rs, en, d4, d5, d6, d7);


int switchState = 0;
int prevswitchState = 0;
int reply;
int POT = A0; //declare POT as arduino input PIN A0
int LED = 9; //declare LED as Arduino output PIN 9
int readValue; // use this variable to look at the 0-5 potentiometer voltage at PIN A0
int writeValue;  // use this variable to write 0-5 V to the lED
float Voltage;
float frequencyon = 10 , frequencyoff = 10; //enter blinking frequency in Hz (on/off time)


void setup()
{
Serial.begin(115200);   //ture the serial port @ 115200


pinMode(LED,OUTPUT);
pinMode(POT,INPUT);
pinMode(switchPin, INPUT);


// The PWM frequency of pin 9 is set at 490 hz, in case you want to change it into one of the following frequencies you uncomment the line you are interested in

//TCCR2B = TCCR2B & B11111000 | B00000001; // for PWM frequency of 31372.55 Hz (31.KHZ)
-----------------
//TCCR2B = TCCR2B & B11111000 | B00000111; // for PWM frequency of 30.64 Hz
-------------------
//TCCR1A = _BV(COM1A1) | _BV(WGM11);               // Enable the PWM output OC1A on digital pins 9
//TCCR1B = _BV(WGM13) | _BV(WGM12) | _BV(CS12);     // Set fast PWM and prescaler of 256 on timer 1
//ICR1 = 62499;// Set the PWM frequency to 1Hz: 16MHz/(256 * 1Hz) - 1 = 62499
lcd.begin(16, 2); // set up the LCD's number of columns and rows:
lcd.clear();

}

void loop()
{


    readValue = analogRead(POT); // read the vcalue from the potentiometer
    writeValue= (255./1023.)*readValue; // do the conversion from 1023 to 255 scale
    analogWrite(LED, writeValue); //value: the duty cycle: between 0 (always off) and 255 (always on). Allowed data types: int
    Serial.print("analog value to Led:");
    Serial.println(writeValue);
    Voltage = (5./255.)* writeValue ;
    delay(5);
    Serial.print ("analog voltage to the LED: ");
    Serial.println (Voltage);


  if ((Voltage < 5) && (Voltage>0.5)) // Exccessing 5 V on the on the PWM pin of the LED signal cable may cause permenant damage, for this reason a threshold is sat at 0.5 (4.5 V)
   {
    lcd.clear();
    lcd.setCursor(0,0);
    lcd.print("v= "); lcd.print(Voltage); lcd.print(" [Volts]");
    lcd.setCursor(0,1);
    lcd.print ("on="); lcd.print(frequencyon); lcd.print(" off="); lcd.print(frequencyoff);
    analogWrite(LED, writeValue);   // turn the LED on (HIGH is the voltage level)
    delay(1000/frequencyon);    // wait for X mSec ( in order to set different frequencies choose between 20, 50, 100, 200 [mSec] to set the frequency to 50, 20, 10, 5 [hz] in order.
    digitalWrite(LED, HIGH);
    delay(1000/frequencyoff); // turn the LED off by making the voltage LOWd


    }


    }

   else  // Voltage higher than 5 or less than .5 V
    {

    lcd.clear();
    lcd.setCursor(0,0);
    lcd.print("v= "); lcd.print(Voltage); lcd.print(" [Volts]");
    lcd.setCursor(0,1);
    lcd.print("!!PROTECTION!!"); // in order to protect the LED from high control voltage
    digitalWrite (LED ,HIGH);
    delay(10000);

      }
      delay(10); // for stabilization
}
```

6.3. Timeline of the project:

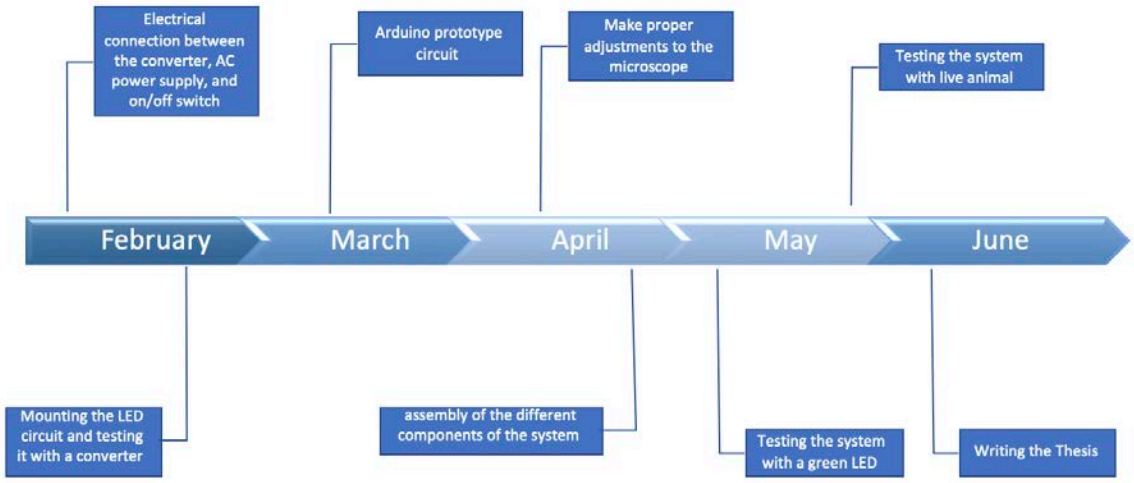


Figure 55: Timeline of the project

6.4. Characteristics of some used components:

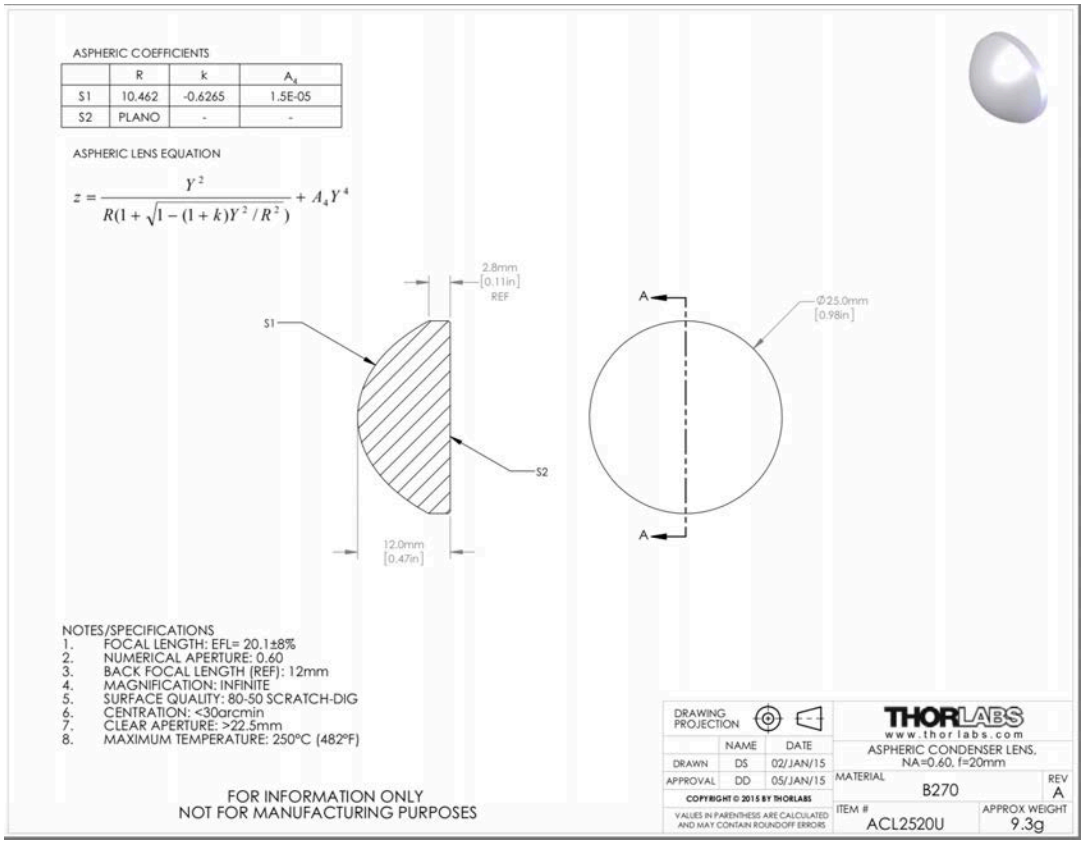
- LED signal pinout

Table 3: Signal pin out	
Pin	**Description**
Pin 1	GND
Pin 2	Analog ADJ
Pin 3	POT
Pin 4	Enable
Pin 5	PWM
Pin 6	Vinx
Pin 7	Thermistor
Pin 8	Thermistor

- Converter (SPN5012) →

MODEL		SPN50-05S		SPN50-12S		SPN50-15S		SPN50-24S		SPN50-48S	
AC Input Voltage		115V	230V	115V	230V	115V	230V	115V	230V	115V	230V
Output Voltage		5		12		15		24		48	
Output Current		10	10	4.3	5	3.5	4	2.2	2.5	1.1	1.1
Input Specification	Efficiency	73%	75%	78%	80%	79%	81%	80%	82%	80%	82%
	Inrush Current 115V	16									
	230V	32									
	Input Current(TYP)	1.1-0.72									
Output Specification	Output Voltage Adjustment Range	±10%									
	Ripple and Noise(m V)	120		150		150		150		200	
	Input Regulation (mV)	10		10		10		20		40	
	Load Regulation (mV)	40		40		40		80		160	
	Temperature Coefficient	0.02%/°C									
	Drift	0.5%+15mV									
	Hold-Up Time 115V	20mS									
	230V	120mS									
	Start-Up Time 115V	100mS									
	230V	100mS									
Other Function	Over Current Protection	Works Over 105% of rating and recovers automatically									
	Over Voltage Protection	Inverter Protection Circute (Works at 115% ~ 150% of rated)									
	Remote Sensing	Built-in									

- Aspheric lens dimensions:

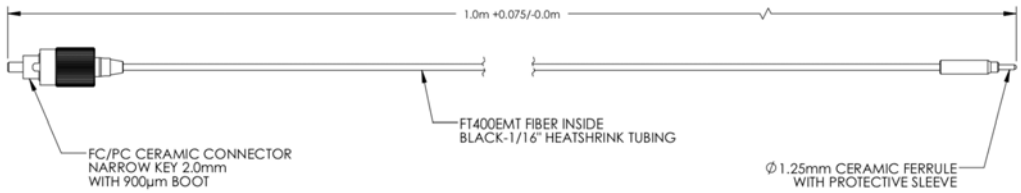


- Optical fiber characteristic:

FIBER SPECIFICATIONS*

ITEM #	CORE DIAMETER	CLADDING DIAMETER	COATING DIAMETER	NA	WAVELENGTH RANGE
FT400EMT	400 ± 8 µm	425 ± 10 µm	730 ± 30 µm	0.39	400 to 2200 nm

*SPECIFICATIONS BASED ON BARE FIBER DATA



INCLUDED BUT NOT SHOWN:
- 2 PROTECTIVE DUST CAPS

FOR INFORMATION ONLY
NOT FOR MANUFACTURING PURPOSES

DRAWING PROJECTION
NAME DATE
DRAWN IR 05/24/2016
APPROVAL BAG 03/21/2019
COPYRIGHT © 2019 BY THORLABS
VALUES IN PARENTHESIS ARE CALCULATED AND MAY CONTAIN ROUNDOFF ERRORS

THORLABS
www.thorlabs.com
1m, 400 µm 0.39 NA FC/PC TO LC FERRULE PATCH CABLE
MATERIAL N/A
REV B
ITEM # M99L01
APPROX WEIGHT 8.6g

- PMT specification:

Specifications

(at +25 °C)

Parameter				H7422-40	H7422-50	Unit
Input Voltage				+11.5 to +15.5		V
Max. Input Voltage for Main Unit				+18		V
Max. Input Current for Main Unit				62		mA
Max. Input Voltage for Thermoelectric Cooler				2.6		V
Max. Input Current for Thermoelectric Cooler				2.2		A
Max. Output Signal Current				2		µA
Max. Control Voltage				+0.9 (Input impedance 100 kΩ)		V
Recommended Control Voltage Adjustment Range				+0.5 to +0.8		V
Effective Area				φ5		mm
Sensitivity Adjustment Range				1: 50		—
Peak Sensitivity Wavelength				580	800	nm
Cathode	Radiant Sensitivity		Typ.	420 nm	108	15	mA/W
				550 nm	176	50	
				800 nm	—	90	
Anode	Standard Type	Radiant Sensitivity *1	Typ.	550 nm	8.8×10^4	2.5×10^4	A/W
		Dark Current *1 *2	Typ.		0.4	0.5	nA
			Max.		1.0	1.3	
	P Type	Radiant Sensitivity *3	Typ.	550 nm	1.8×10^5	5.0×10^4	A/W
		Dark Count *2 *3	Typ.		100	125	s^{-1}
			Max.		300	375	
Rise Time *1			Typ.		1.00		ns
Ripple Noise *1 *4 (peak to peak)			Max.		0.6		mV
Settling Time *5			Typ.		0.2		s
Operating Ambient Temperature *6				+5 to +35		°C
Storage Temperature *6				-20 to +50		°C
Weight				Approx. 400		g

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